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Theodore Caldwell Janeway

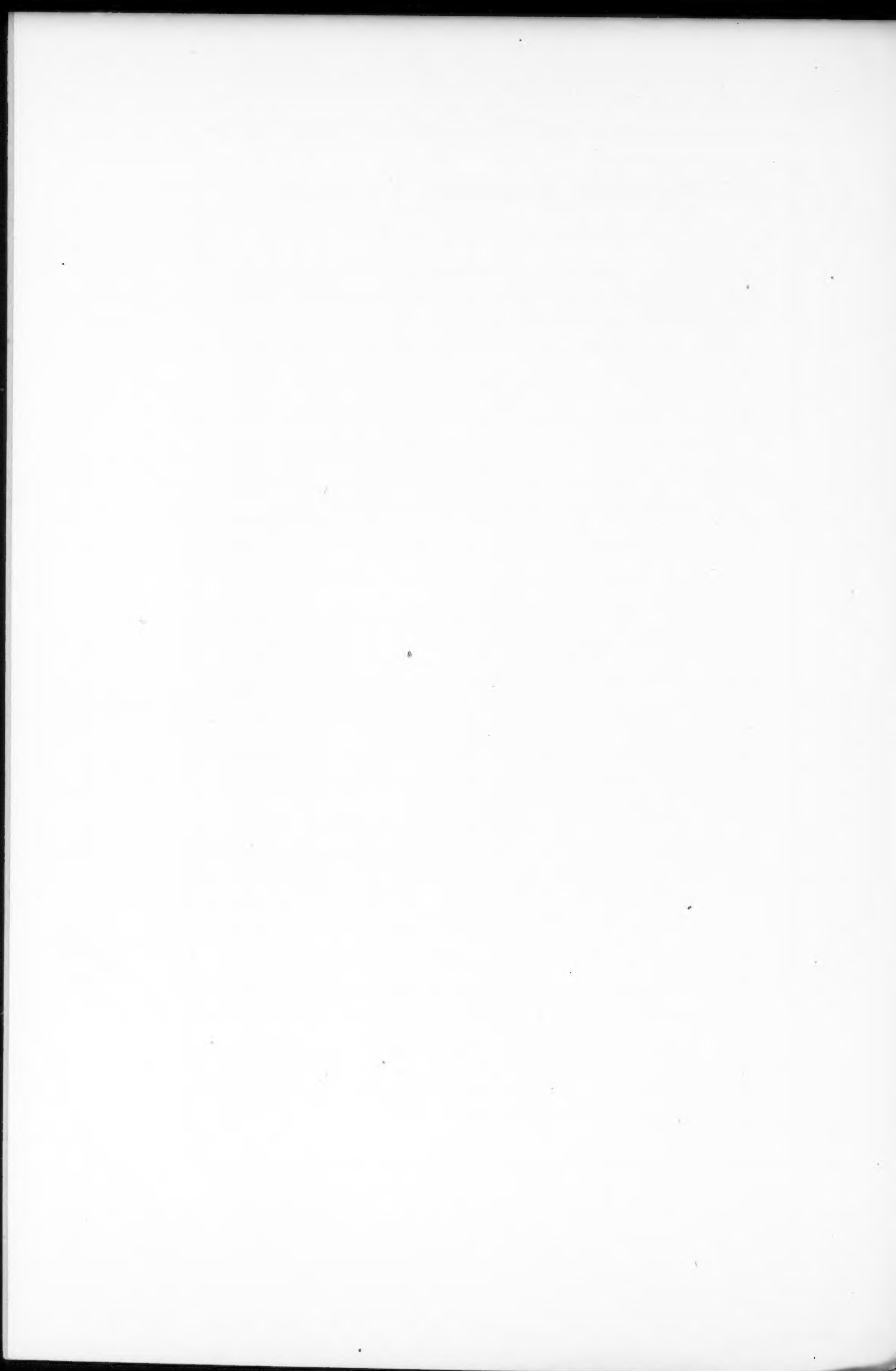
Born 1872 Died 1917

At a meeting of the Board of Scientific Directors of The Rockefeller Institute for Medical Research, held on January 19, 1918, the following Minute was adopted:

RESOLVED, *That the Scientific Directors of The Rockefeller Institute record their profound sense of loss in the death of their honored and beloved associate,* THEODORE CALDWELL JANEWAY, M.D., *who has served on the Board with devoted zeal since his election to succeed Dr. Christian A. Herter in 1911. Dr. Janeway at the height of his powers and in the midst of the most productive period of his life was stricken with pneumonia while in active service in the Medical Corps of the Army, to which, since the United States entered into war with Germany, he gave invaluable and unmeasured service. His life was sacrificed to patriotic duty rendered to his country without reserve. Dr. Janeway's period of office on the Board of Scientific Directors of The Rockefeller Institute was restricted to a brief seven years, yet its importance was very great, as he brought to its service learning, keen intelligence, and broad vision.*

Dr. Janeway was a highly skilled and widely read clinician, and he was also a notable exponent of the scientific method in internal medicine. A graduate of the Sheffield Scientific School and of the College of Physicians and Surgeons, he emphasized the importance of chemistry and physics, the two sciences on which he based his clinical conceptions. Coming early under the mature and wise influence of his distinguished father, he received from him the more purely clinical and pathological impress which so much contributed to his broader development. In rapid succession Dr. Janeway became instructor in medicine at New York University and Bellevue Hospital Medical College in 1905, associate professor and then professor of medicine at Columbia University in 1909. During this period, in 1907, he was instrumental in founding the Russell Sage Institute of Pathology, which throughout its connection with the City Hospital was made a valuable adjunct to the courses in medicine which he conducted. It was natural and logical, because of the work he had done in internal medicine, that Dr. Janeway should be called to fill the full time chair in internal medicine established at the Johns Hopkins Medical School in 1914. The acceptance of the new professorship was made at a large financial sacrifice, but his altruistic action was wholly consonant with the broad and sympathetic attitude which he always held toward medical teaching and research.

Dr. Janeway's untimely death cut short not only a career in medicine which he had inaugurated with every promise of distinguished success, but has at the same time deprived The Rockefeller Institute of one of its ablest and wisest counsellors, and the medical profession of a great physician.



A STUDY OF THE LOW BLOOD PRESSURES ASSOCIATED WITH ANAPHYLACTIC AND PEPTONE SHOCK AND EXPERIMENTAL FAT EMBOLISM, WITH SPECIAL REFERENCE TO SURGICAL SHOCK.

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PLATES 17 TO 21.

(Received for publication, November 28, 1917.)

In a preliminary report[1] attention was called to certain fundamental differences between the low blood pressures associated with peptone shock and experimental fat embolism. Consideration of these differences is important because they have a direct bearing upon certain theories as to the etiology and mechanism of surgical shock. It is proposed in this paper to give in detail the experimental data upon which some of the statements made in that report were based, to add new observations, and to discuss the results obtained. The theories concerned are that surgical shock is due to loss of vascular tone in the splanchnic region, that it is due to loss of peripheral vascular tone, and that it is due to fat embolism.

Mann[2] is of the opinion that "the cause of shock is an actual loss of red cells and fluid from the circulating blood through stasis, diapedesis, exudate, and endothelial changes brought about by the reaction of the great delicate splanchnic area to irritation." Janeway and Jackson[3] concluded from their study of low blood pressures induced by compression of the inferior vena cava, that the dilatation of the peripheral venules and capillaries as a result of the increased venous pressure caused such a loss of tone, that even after normal pressure was restored by release of the compression, they became overfilled with blood. As a

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[1] Simonds, J. P., *J. Am. Med. Assn.*, 1917, lxix, 883.

[2] Mann, F. C., *Bull. Johns Hopkins Hosp.*, 1914, xxv, 205.

[3] Janeway, H. H., *Proc. Soc. Exp. Biol. and Med.*, 1914–15, xii, 83. Janeway, H. H., and Jackson, H. C., *ibid.*, 1914–15, xii, 193.

result of this, the arterial pressure fell to a very low level and the animal died in a few hours. Warthin,[4] Bissell,[5] and Porter[6] are convinced that fat embolism is "a cause" of surgical shock.

Technique.

All the experiments here reported were made upon dogs under ether anesthesia. Altogether, more than thirty-five animals have been used in the study of the problems involved. The arterial pressure was taken from the carotid artery according to the usual technique. The fat, in the form of neutral olive oil, was injected through a cannula in the femoral vein, being washed into the vessel with 5 or 6 cc. of isotonic salt solution. Witte's peptone (0.5 to 1 gm., according to the size of the dog) was dissolved in 10 cc. of salt solution and injected through the same cannula. Standard doses of nicotine (1 cc. of a 1:4,000 solution) and of adrenalin (1 cc. of a 1:50,000 solution) were also administered intravenously in the same manner.

In making a record of the venous pressure the following method was employed. The external jugular vein was exposed as low down in the neck as possible. Into it a wide cannula with a large bulb completely filled with 10 per cent solution of sodium carbonate was inserted. The long proximal end of the cannula, measuring 4 to 6 cm., was pushed well down into the subclavian vein and even in some instances into the superior vena cava. Before inserting the cannula into the vessel, a rubber tube was attached to the distal end and closed with a screw clamp to prevent the carbonate solution from running into the vein. The cannula was then connected by rubber and glass tubing (including a T-tube) with a manometer of the type described by Hoskins and Gunning.[7] All connections were made airtight, because air transmission was used. In the manometer there was a solution of zinc chloride with a specific gravity of 1.36; that is, one-tenth the specific gravity of mercury.[8] The float in the manom-

[4] Warthin, A. S., *Internat. Clinics*, 1913, iv, series 23, 171.

[5] Bissell, W. W., *Surg., Gynec. and Obst.*, 1917, xxv, 8.

[6] Porter, W. T., *Boston Med. and Surg. J.*, 1917, clxxvi, 248.

[7] Hoskins, R. G., and Gunning, R. E. L., *Am. J. Physiol.*, 1917, xliii, 298.

[8] For the preparation and the accurate measurement of the specific gravity of this solution I am indebted to Professor J. H. Long.

eter was attached by a thread to the longer branch of a light heart lever exactly half way between the fulcrum and the writing point. After all the connections had been made, it was necessary to produce a slight negative pressure in the apparatus. This was readily done through the T-tube. The screw clamp at the cannula was then cautiously released. The apparatus was so adjusted that the column of liquid forced out of the cannula in high venous pressures did not rise above the level of the vein. In other words, the column of liquid was kept parallel with the axis of the vessel. The respiratory and other changes in the venous pressure were recorded as shown in Figs. 1, 2, and 3. The change in position of the writing point of the heart lever represented ten times the change in pressure expressed in millimeters of mercury, because the fluid in the manometer had a specific gravity one-tenth that of mercury.

EXPERIMENTAL.

The three following protocols of experiments are typical. All operative procedures were carried out under ether anesthesia, and the animal was killed at the end of the experiment without recovery from the anesthetic. A cannula was placed in the trachea and connected with an ether bottle. The remainder of the technique has been sufficiently described above.

Dog 1.—Male; weight 18 pounds. October 15, 1917.

10.07 a.m. Blood pressure 130 mm. of mercury.

10.07 to 10.09 a.m. 12 cc. of olive oil injected in doses of 2 cc. each.

10.10 a.m. Blood pressure 110 mm.

10.13 a.m. Blood pressure 130 mm.

10.13 to 10.15 a.m. 8 cc. of olive oil injected in doses of 2 cc. each.

10.14 a.m. Blood pressure 125 mm.

10.16 a.m. Blood pressure 105 mm.

10.20 a.m. Blood pressure 95 mm. 4 cc. of olive oil injected.

10.21 a.m. Blood pressure 80 mm.

10.25 a.m. Blood pressure 70 mm. 2 cc. of olive oil injected.

10.26 a.m. Blood pressure 65 mm. 2 cc. of olive oil injected.

10.27 a.m. Blood pressure 50 mm. Respiration ceased. Artificial respiration started. Heart stopped beating at 10.29 a.m.

Autopsy.—Dilatation of the right side of the heart; left side contains small amount of blood. General venous stasis. Very slight edema of the lungs.

Dog 2.—Male; weight 14 pounds. June 29, 1917.

1.20 p.m. Blood pressure 180 mm. of mercury. 1 gm. of Witte's peptone injected intravenously. Blood pressure fell within 30 seconds to 55 mm.

1.40 p.m. Blood pressure 130 mm.

1.50 p.m. Blood pressure 170 mm.

1.50 to 1.52 p.m. Three injections of olive oil, 2 cc. each.

1.53 p.m. Blood pressure 170 mm. 2 cc. of olive oil injected. Respiration became slow and shallow and blood pressure began to decline very slowly.

1.57 p.m. Blood pressure 120 mm. Respiration continued to become slower and slower. Blood pressure fluctuated but had a general downward tendency. Reaction to adrenalin and nicotine normal.

2.25 p.m. Blood pressure 70 mm. Respiration slow and labored.

2.40 p.m. Blood pressure 85 mm.

2.55 p.m. Respiration stopped entirely. Blood pressure 50 mm. Artificial respiration begun and ether removed. Blood pressure steadily rose during the artificial respiration until 3.05 p.m.

3.05 p.m. Blood pressure 95 mm. Respiration became spontaneous and artificial respiration was stopped. Immediately upon stoppage of artificial respiration the pressure rose rapidly.

3.10 p.m. Blood pressure 140 mm. Ether given again because of return of corneal reflex. Respiration again became slow and labored. During the next 50 minutes the blood pressure fluctuated between 140 and 70 mm.

4.05 p.m. Blood pressure 90 mm. 5 cc. of olive oil injected. Blood pressure fell to 80 mm.

4.06 p.m. Blood pressure 90 mm. 5 cc. of olive oil injected. Blood pressure fell to 75 mm.

4.07 p.m. Blood pressure 80 mm. 3 cc. of olive oil injected. Blood pressure fell to 70 mm.

4.08 p.m. Blood pressure 70 mm.

4.09 p.m. Blood pressure 60 mm.

4.10 p.m. Blood pressure 55 mm. Respirations very slow and shallow.

4.11 p.m. Blood pressure 40 mm. Respiration stopped.

4.13 p.m. Heart stopped.

Dog 3.—Male; weight 42 pounds. October 18, 1917.

11.33 a.m. Blood pressure 130 mm.

11.36 a.m. Blood pressure 125 mm. 1 gm. of Witte's peptone injected.

11.37 a.m. Blood pressure 55 mm. Venous pressure fell during this time approximately 14 mm.

11.47 a.m. Blood pressure 120 mm. Venous pressure at its former level.

11.47 a.m. to 12.08 p.m. 40 cc. of olive oil injected in doses of 5 cc. each.

12.08 p.m. Blood pressure 110 mm. The reactions of the venous and arterial pressures to the injections of oil are shown graphically in Fig. 3.

12.10 to 12.11 p.m. 10 cc. of olive oil injected in doses of 5 cc. each.

12.12 p.m. Blood pressure 105 to 110 mm.
12.13 p.m. 5 cc. of olive oil injected.
12.14 p.m. Blood pressure 95 to 100 mm. The pressure steadily declined and heart stopped beating at 12.16 p.m. During the fall of arterial pressure the venous pressure constantly rose (Fig. 3).

Autopsy.—Dilatation of the right side of the heart; left side contains very little blood. General venous stasis. Slight edema of the lungs.

The characteristic feature of anaphylactic and peptone shock in the dog is a marked and abrupt fall in arterial blood pressure. Experiments by Edmunds,[9] Manwaring,[10] Denecke,[11] Jaffé and Přibram,[12] and Weil[13] have shown that the liver is an essential element in the production of this type of shock in this animal[14] (Figs. 1 and 4). Although Robinson and Auer,[15] by means of the electrocardiograph, found disturbances in the conduction of the heart and abnormalities in the ventricular contractions, it is evident that the action upon the heart is of relatively minor importance. The cause of the low blood pressure is a stagnation of the blood in the liver and splanchnic region which prevents the right heart from receiving sufficient blood to keep the left ventricle supplied with an amount adequate to maintain the arterial pressure at its normal level.[16]

The studies of blood pressures in experimental fat embolism reported in the literature are meager and for the most part give too few details to be satisfactory. Warthin[4] injected 7 cc. of olive oil directly into the heart of a dog, and observed a rapid fall in arterial pressure and an increase in pressure in the auricle and jugular vein, "as shown in the charts." But no "charts" appear in the article. He states further that "repeated injections cause large systolic pulsations in the right auricle, the arterial pressure steadily falls, and that in the auricle and jugular steadily goes up. Finally there is delirium cordis and death." The weights of

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[9] Edmunds, C. W., *Z. Immunitätsforsch., Orig.*, 1913, xvii, 105; 1914, xxii, 181.
[10] Manwaring, W. H., *Z. Immunitätsforsch., Orig.*, 1911, viii, 1.
[11] Denecke, G., *Z. Immunitätsforsch., Orig.*, 1913–14, xx, 501.
[12] Jaffé, R. H., and Přibram, E., *Virchows Arch. path. Anat.*, 1915, ccxx, 213.
[13] Weil, R., *J. Immunol.*, 1917, ii, 525.
[14] Loewit, M., (*Arch. exp. Path. u. Pharm.*, 1913, lxxiii, 1) has pointed out certain alleged differences between anaphylactic and peptone shock.
[15] Robinson, G. C., and Auer, J., *J. Exp. Med.*, 1913, xviii, 556.
[16] Eisenbrey, A. B., and Pearce, R. M., *J. Pharm. and Exp. Therap.*, 1912–13, iv, 21.

the animals, the quantities of oil used in the "repeated injections," and the time allowed to elapse between them are not stated.

Bissell[5] gives one chart showing the arterial and venous pressures in a dog weighing 7.4 kilos (16.3 pounds) which received 17 cc. of olive oil in doses of 7, 5, and 5 cc. within 2 minutes. After the first injection (of 7 cc.), there was only a "volume change" in arterial and venous pressure; after the second, there was a very transient fall in arterial pressure and an equally transient rise in venous pressure; after the third injection, the venous pressure went up very abruptly while the arterial pressure gradually fell and the animal quickly died.

Porter[6] mentions experiments upon eight cats. One received 3 cc. of "officinal emulsion" of olive oil injected slowly into the jugular vein. Very soon there followed a fall in carotid pressure. In two other experiments thick cream was used, and in the remainder, olive oil. "2 to 4 cc. of olive oil in a large cat has never failed to produce a fall in blood pressure to one-half or less the normal level." In one experiment the diastolic pressure fell from 140 to 65 mm. of mercury and later to 40 mm. "In this cat the tracing showed that the fall in blood pressure could not be ascribed to changes in the heart beat. The same is usually true when the injection is not made too rapidly. The clinical picture is essentially that of traumatic shock in human beings."

It appears that in the above instances cited from the literature relatively large amounts of oil in proportion to the body weight of the animal were injected within the space of a few minutes. Graham,[17] on the other hand, made repeated small injections of small amounts of olive oil into the veins of rabbits over a period of days, but no blood pressure tracings were taken.

The presence of fat embolism in some cases of surgical shock has been established by the work of Bissell.[5] But it has also been found by Flournoy,[18] and by Katase,[19] in a variety of other pathological conditions.

In experimental fat embolism, the fall in blood pressure is gradual and progressive. But the beginning of the fall in pressure is not synchronous with the beginning of the injections of the oil. A remarkably large amount of oil can be introduced into the veins of an anesthetized dog without producing more than a very slight and temporary fall in arterial pressure. There appears to be a roughly quantitative relation between the body weight of the dog and the amount of olive oil that must be injected to cause the arterial pressure to start downward permanently. In the experiments of this series it was

[17] Graham, G. S., *J. Med. Research*, 1907, xi, 459.

[18] Flournoy, T., Contribution à l'étude de l'embolie graisseuse, Inaugural dissertation, Strassburg, 1878, quoted by Warthin.[4]

[19] Katase, A., *Cor.-Bl. schweiz. Aertze*, 1917, xlvii, 545.

necessary to inject approximately 1 cc. of oil for each pound of body weight in order to bring about a fall of pressure of any duration. The results in twelve animals are shown in tabular form in Table I. In this table are shown the weight of the animal and the number of cubic centimeters of oil that were injected before the arterial pressure was permanently and materially lowered. It was somewhat difficult to establish a standard. But for the purposes of this tabulation, it was considered that the pressure had been permanently and materially reduced when there had been a lasting fall of 20 to 30 mm. of mercury from the initial pressure, provided the decline did not reduce the pressure below 100 mm. of mercury. The minimum was 29 cc. for a 35 pound dog; the maximum, 70 cc. for a 30 pound dog. If we omit from consideration Dog 13, which was distinctly abnormal in his resistance to the injections of oil, it is seen that the average quantity of oil necessary to initiate a permanent fall in blood pressure in the dog is approximately equal to 1 cc. of oil for each pound of weight of the

TABLE I.

The Quantity of Oil Necessary to Reduce Permanently and Materially the Arterial Pressure in Anesthetized Dogs.

Animal No.	Weight.	Amount of oil necessary to reduce arterial pressure.	Amount of oil per pound of body weight.	Dosage of oil injections.	Frequency of injections.	Total amount of oil injected.	Initial injection of Witte's peptone.
	lbs.	*cc.*	*cc.*	*cc.*	*min.*	*cc.*	
4	19	21	1.1	2–5	1–2	26	No injection.
5	35	29	0.8	2	2–4	37	" "
6	24	23	0.9	2	3–4	23	" "
7	34	35	1.0	5	1–5	55	Injection.
8	27	28	1.0	2	2	28	No injection.
9	30	42	1.4	2	1	44	" "
10	30	25	0.8	2–4	1	38	Injection.
1	18	20	1.1	2	1	28	No injection.
3	42	50	1.2	5	1–3	55	Injection.
11	15	16	1.0	1–2	1	19	"
12	18	18	1.0	2	1–2	18	"
13	30	70	2.3	2–10	1–9	70	No injection.
Average (omitting Dog 13) . .			1.0+				

animal; that is, a 25 pound dog would require about 25 cc. of oil, and a 150 pound man about 150 cc., to induce a lasting fall in pressure, if the same quantitative relations hold for man as for the dog.

It apparently makes only slight difference whether this critical quantity is injected in amounts of 1 or 2 cc. of oil at intervals of 1 or 2 minutes, or in quantities of 5 to 10 cc. at a time. When the larger amounts are injected, with short intervals between the injections, the mechanism of adaptation is put to a greater strain, the critical point may be slightly lowered, the immediate fall is more marked, and the recovery more slow (Fig. 5). But even when relatively enormous quantities of oil are injected at one time and fairly rapidly (for example, in one experiment, 75 cc. of oil were injected very rapidly into a 29 pound dog from a burette connected with the cannula in the femoral vein) the fall in pressure is much more gradual than in the case of peptone or anaphylactic shock (Fig. 6).

Several factors of safety, which in peptone shock appear to be entirely useless, are thoroughly effective in fat embolism in the dog. In the first place, many years ago, Lichtheim[20] showed that three-fourths of the pulmonary circulation may be occluded without affecting the systemic pressure. Tigerstedt[21] and Gerhardt[22] have both confirmed this observation, although Gerhardt, who worked with spontaneously breathing rabbits, questioned Lichtheim's explanation of the phenomenon. More recently, Kuno[23] has shown that the lungs may contain from 8.8 to 19.44 per cent of the amount of blood in the body, depending upon the condition of the circulation.

It is evident, therefore, that much of the vascular space of the lungs can be closed without causing a fall in arterial pressure, a conclusion amply justified by the results here reported. This is due in part to the presence of an excessively large vascular area in the lungs in which there is probably ordinarily much dead space where the circulation is not active. But the occlusion may involve more than this

[20] Lichtheim, L., Die Störungen des Lungenkreislaufes und ihr Einfluss auf den Blutdruck, Breslau, 1876, cited by Tigerstedt, R., *Ergebn. Physiol.*, 1903, ii, pt. 2, 528.

[21] Tigerstedt, R., *Ergebn. Physiol.*, 1903, ii, pt. 2, 528; *Skand. Arch. Physiol.*, 1903, xiv, 259.

[22] Gerhardt, D., *Z. klin. Med.*, 1904, lv, 195.

[23] Kuno, Y., *J. Physiol.*, 1917, li, 154.

excess of vascular area, and the systemic pressure may still be maintained at the normal level by increased work on the part of the right ventricle. Gerhardt[22] observed a rise in pressure in the pulmonary artery under these conditions. Furthermore, the capillaries of the lungs are capable of very great distention, and by dilatation the still unoccluded capillaries can permit the passage of an undiminished or only slightly diminished supply of blood to the left side of the heart.

A more detailed analysis of the curve of arterial pressure obtained during the successive injections of small amounts of olive oil into the veins of a dog shows it to be more complex than the simple curve of peptone shock. In Fig. 2 is shown the effect of injecting a 15 pound dog with 1 or 2 cc. doses of olive oil at intervals of 1 to 2 minutes, until a total of 19 cc. had been injected. It is seen that the first one or two injections produced hardly any perceptible effect. With each succeeding injection the immediate fall in pressure became greater and greater and the return to normal slower and slower. Finally, a point was reached at which, after each injection, the pressure did not again reach its former level. In the case of this particular animal, an injection of peptone had been given, from the effects of which it had not completely recovered when the injections of oil were begun. This accounts for the fact that the arterial pressure continued to rise slowly for some minutes in spite of the injections of oil (see also Fig. 3).

After the critical point had been reached, each succeeding dose of oil brought the pressure lower and lower. If the injections were stopped soon enough, however, the animal could usually be kept alive for some time and the various phases of the condition studied. In all the animals, when the arterial pressure showed a permanent and material depression, however slight at first, the condition gradually became worse, and the animal died in from 1 to 3 hours. If the injections were continued, the animal succumbed very quickly. The anesthetic doubtless played a part in the progressively fatal course.

The interpretation of these results is, perhaps, obvious. It would seem to be a legitimate conclusion that the oil first injected merely filled some of the excess vascular space in the lungs. When this excess was used up, the unoccluded capillaries dilated sufficiently to permit the passage of an undiminished or only slightly diminished

volume of blood. When the capillary area was still further reduced, the right ventricle was still able, by increasing its work, to deliver the necessary amount of blood to the left side of the heart. It is not to be supposed, however, that each of these factors of safety came into action separately and in the order named. They probably all became active early, but in varying degrees. The increasing extent of fall and slower recovery after each succeeding injection was due to the increasing difficulty of readjustment of these various factors to the augmented load placed upon them. A point was ultimately reached at which the fall in pressure induced by each injection was roughly proportional to the amount of reduction in the remaining vascular space in the lungs by that injection. That this was probably true was indicated by the great difference in the amount of fall in pressure induced by the same volume of oil injected before and after the critical quantity had been reached (Figs. 2 and 3). The progressively downward course was probably the result of a break in compensation on the part of the various adaptive factors of safety.

Differences in the Effect of Peptone Shock and Experimental Fat Embolism upon Venous Pressure.

The differences in the effects of peptone shock and experimental fat embolism upon venous pressure are equally striking. In the former, there is usually a slight preliminary rise due to the volume of fluid suddenly injected into the veins with the peptone. This is followed by a precipitate fall. The arterial and venous pressures thus run practically parallel (Fig. 1). While the arterial pressure is gradually rising as the animal recovers, the venous pressure also rises. The onset of dyspnea during peptone shock causes a further fall in venous pressure and a rise in arterial pressure as already described.

In experimental fat embolism, the venous and arterial pressures change in opposite directions. During the successive injections of small amounts of oil there occurs usually a slight temporary rise in venous pressure simultaneously with the slight evanescent fall in arterial pressure. But both quickly return to normal. It is not until the critical quantity of oil has been injected that there is a lasting rise in venous pressure. A relatively small amount of oil in-

jected at this time will cause a rapid rise in venous pressure synchronous with the marked fall in arterial pressure described above (Figs. 2 and 3).

In peptone shock the venous pressure falls because of the sudden stagnation of blood in the liver and organs of the splanchnic area. It does not reach the larger veins which are relatively collapsed. In fat embolism the blood cannot reach the systemic circulation because of the blocking of its passage through the lungs. The blood of the body, therefore, accumulates in the veins. Since all the vessels still retain their normal tone, the venous pressure rises.

It seems obvious that if a sufficient amount of fat has entered the lungs to cause a fall in arterial pressure in any case of surgical shock, there should also be present at the same time an elevation of venous pressure, for in experimental fat embolism in the dog the venous pressure begins to rise only when the arterial pressure begins to fall. There has not been opportunity to make observations upon this point on human cases of shock.

Effects of Dyspnea in Peptone Shock and in Experimental Fat Embolism.

In two papers published in 1916, it was shown that dyspnea will cause a rise in arterial pressure in anaphylactic and peptone shock.[24] Weil,[13] who appears to have seen only the first of these papers in which the idea was not fully developed, has recently stated that "this novel hypothesis is quite unsupported by confirmatory experiments." It seems advisable, therefore, to restate briefly the evidence upon which this claim was made. The phenomenon was first observed in the course of experiments to determine the state of vasomotor irritability in anaphylactic shock in dogs. It was noticed that during the stage of low blood pressure, doses of 1 cc. of a 1:4,000 solution of nicotine sometimes produced a greatly exaggerated reaction. When this occurred the percentile rise[25] varied from 30 per cent to more than 200 per cent, as contrasted with a percentile rise of 10 to 17 per cent before the induction of shock, and with an average percentile rise of less than 10 per cent after its production in those instances in which nicotine did not bring about dyspnea (Table II). It was observed further, that whenever this augmented reaction occurred, the injection of nicotine was invariably followed by more or less dyspnea, and that the rise in pressure was roughly proportional to the severity of the dyspnea.

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[24] Simonds, J. P., *J. Infect. Dis.*, 1916, xix, 746; *Arch. Int. Med.*, 1916, xviii, 848.
[25] Porter, W. T., *Am. J. Physiol.*, 1907–08, xx, 399; 1914, xxxiii, 373.

Dyspnea induced by other means, such as increasing the amount of carbon dioxide in the inspired air, and stimulation of an afferent nerve, has given similar results. But there is no method of causing dyspnea in an anesthetized dog that is entirely free from criticism. Asphyxia is known to cause a rise in blood pressure in the normal anesthetized animal. In the stimulation of an afferent nerve it is not always easy to determine for a given animal the exact strength of current that will produce the maximum of dyspnea with a minimum of pressor or depressor effect.

There is reason to believe that for the purpose of determining the effect of dyspnea upon the low blood pressure associated with peptone shock, that induced by the injections of nicotine may be freer from criticism than that brought about by any other method. It has been shown by Hoskins and Ranson[26] that nicotine causes vasoconstriction chiefly by its action upon the sympathetic ganglia, but partly by its action upon the vasomotor center. Adrenalin, on the other hand, produces a rise in blood pressure as a result of its action upon the nerve endings in the vessel wall.[27] Hence nicotine, as a rule, cannot produce a rise in blood pressure in those conditions in which adrenalin is completely ineffective. In anaphylactic shock and in peptone poisoning, injections of adrenalin are without appreciable effect until the blood pressure has begun to rise. Even when the reaction does return, the percentile rise is reduced, and only gradually approaches the normal for the given animal as the absolute pressure nears its former level. The reaction to nicotine, when unaccompanied by dyspnea, follows an exactly similar course; that is, the actual and percentile rises in blood pressure following nicotine injections (without dyspnea) gradually increase from zero to their pre-shock values as the absolute pressure rises to its normal, as shown in Table II. This is especially evident when the results of injections of nicotine in spontaneously breathing animals are compared with those obtained in dogs similarly treated but with the thorax open and artificial respiration employed. The fact that the actual and percentile rises caused by injections of adrenalin and nicotine, when

[26] Hoskins, R. G., and Ranson, S. W., *J. Pharm. and Exp. Therap.*, 1915, vii, 375.

[27] Dixon, W. E., *J. Physiol.*, 1904, xxx, 97.

TABLE II.

Effects of Injections of Nicotine with and without Dyspnea upon Low Blood Pressure Associated with Anaphylactic and Peptone Shock.*

Animal No.	Before induction of peptone or anaphylactic shock.				After induction of peptone or anaphylactic shock.				
	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Dyspnea.
	mm.	*mm.*	*mm.*	*per cent*	*mm.*	*mm.*	*mm.*	*per cent*	
14	110	125	15	10	35	35	0	0	None.
					35	120	85	243	Violent.
					35	75	40	114	Marked.
15	105	115	10	10	15	20	5	33	Slight.
					35	90	55	157	Marked.
					75	80	5	7	None.
					75	115	40	53	Moderate.
16	125	145	20	16	30	30	0	0	None.
					50	85	35	70	Moderate.
					80	120	40	50	"
17	140	150	10	7	35	70	35	100	Marked.
					20	70	50	250	Violent.
					80	115	35	44	Moderate.
18	180	200	20	11	35	38	3	9	None.
					30	40	10	33	Slight.
					55	100	45	80	Moderate.
19	100	110	10	10	20	25	5	25	Marked.
					35	50	15	43	"
					45	65	20	44	Moderate.
					55	65	10	18	None.
					60	80	20	33	Slight.
					55	65	10	18	None.
20	160	175	15	9	30	30	0	0	None.
					35	45	10	28	Slight.
					55	65	10	18	None.
					60	120	60	100	Marked.

* Standard dose, 1 cc. of a 1: 4,000 solution of nicotine intravenously.

TABLE II—*Concluded.*

Animal No.	Before induction of peptone or anaphylactic shock.				After induction of peptone or anaphylactic shock.				
	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Initial pressure.	Maximum resultant pressure.	Absolute rise in pressure.	Percentile rise in pressure.	Dyspnea.
	mm.	*mm.*	*mm.*	*per cent*	*mm.*	*mm.*	*mm.*	*per cent*	
21	100	110	10	10	55	55	0	0	Chest open. Artificial respiration.
					45	47	2	4	
					65	70	5	8	
					52	56	4	8	
					65	70	5	8	
22	120	140	20	17	25	25	0	0	Chest open. Artificial respiration.
					50	52	2	4	
					65	70	5	8	
					85	95	10	12	
					105	120	15	14	

unaccompanied by dyspnea, in anaphylactic and peptone shock run parallel courses, indicates that the direct pressor action of nicotine is inhibited by the same condition that renders adrenalin ineffective. It is for these reasons that in anaphylactic and peptone shock we consider the exaggerated rise in blood pressure induced by nicotine as a more purely mechanical effect than a similar rise accompanying dyspnea brought about by any other method.

We may summarize the evidence that the augmented reaction to nicotine frequently observed in the condition of low blood pressure associated with peptone poisoning is due wholly to the mechanical effect of the dyspnea, as follows: (1) It occurs only in the stage of low blood pressure, and only when the dose of nicotine causes dyspnea. It does not appear when the blood pressure is normal even if dyspnea is produced. (2) It has not been observed in any animal in which the chest has been opened and artificial respiration employed. (3) It is not due to any cumulative effect because of the slowed circulation, for a double dose of the drug will not cause such an augmented reaction before the condition of shock is induced. During the condition of shock a 2 cc. dose is more likely to produce dyspnea and is therefore more frequently followed by a magnified rise in pressure

than is a 1 cc. dose. A double dose without dyspnea does not yield the normal, *i.e.* pre-shock, percentile rise. (4) This augmented reaction occurs at a stage in the condition of shock when adrenalin is without effect, and when, from our knowledge of the pharmacologic action of nicotine,[26] no response to injections of that drug is to be expected. (5) With a large reservoir of stagnating blood in the liver, conditions are favorable for the most effective results from increased respiratory suction and from the force-pump action of the vigorously contracting diaphragm. The distance from the right auricle to the point of entry of the hepatic vein into the inferior vena cava is short. The latter vessel is prevented from collapsing by its attachment to the central tendon of the diaphragm through which it passes. By this pump-like action increased amounts of blood are brought to the underfilled right ventricle and delivered at once through the unimpeded pulmonary circulation to the left ventricle and thence to the systemic circulation, with the resultant rise in pressure. When the blood pressure is normal, dyspnea does not cause this marked rise because there is no large convenient reservoir of blood for respiratory suction to act upon, and because the right side of the heart is already filling properly with each diastole.

During the first ½ minute or so after the blood pressure approaches or reaches its minimum in anaphylactic or peptone shock, dyspnea frequently is not effective in causing a rise in pressure. This is especially well seen by contrasting the results of injections of nicotine in Dogs 17 and 19 (Table II). With a pressure of 20 mm. of mercury immediately before the injection of the second dose of nicotine (11 minutes after injecting 5 cc. of normal horse serum) in Dog 17, a violent dyspnea produced a rise of 250 per cent. With the same initial pressure in Dog 19, between ½ and 1 minute after giving 5 cc. of horse serum, a rise of only 25 per cent was produced in spite of the marked dyspnea which the injection of nicotine caused.

The type of the rise in blood pressure which accompanies dyspnea in peptone shock varies with the severity of the dyspnea. If this is violent, as in Dog 14 (Fig. 4), the rise is rapid; if only moderate, as in Dog 17, the rise is somewhat less steep.[28] The degree of dyspnea does

[28] The tracing from this dog was published in connection with the paper on "Anaphylactic shock in dogs," *J. Infect. Dis.*, 1916, xix, 746.

not affect the percentile rise so much as it affects the rate at which the rise occurs. In either case, there is, in the early stages, a tendency for the pressure to fall again, but usually less rapidly than it rose. As a rule, the pressure does not again return to its former low level, unless the dose of peptone was very large. It then not infrequently falls to a still lower plane. For example, Dog 17, with a blood pressure of only 20 mm. of mercury at the time of the induction of the second period of dyspnea, by nicotine, was apparently *in extremis.* The dyspnea was violent and induced a rise in pressure of 250 per cent, and the animal recovered in a relatively short time. Similar results, although usually less spectacular, have been obtained in many animals.

Dyspnea is, therefore, an important therapeutic agent in low blood pressures of the type present in anaphylactic and peptone shock. By bringing the pressure above the danger zone at frequent intervals by the repeated induction of short periods of dyspnea, the life of the animal can usually be saved. Whether equally beneficial results will follow its use in surgical shock in human patients will depend upon the mechanism of that condition. If the low blood pressure in surgical shock resembles that in peptone poisoning in that it is accompanied by a reservoir of stagnating blood in the liver, it would seem reasonable to expect salutary results from the frequent induction of periods of dyspnea by some method which can be applied with safety in human cases, such as increasing the carbon dioxide content of the inspired air. If, on the other hand, surgical shock has an entirely different mechanism, as, for example, fat embolism, little permanent benefit can be expected from the use of dyspnea. Incidentally it may be remarked that the study of the effects of dyspnea in surgical shock in man may yield information of value in determining the nature and mechanism of that condition.

Since I had observed that dyspnea causes a rise in blood pressure in anaphylactic and peptone shock,[24] Porter's[29] report that dyspnea causes a rise in the low blood pressure accompanying fat embolism led to the present comparative study of these two conditions. My experiments have confirmed Porter's observation, although the re-

[29] Porter, W. T., *Boston Med. and Surg. J.*, 1917, clxxvi, 699.

sults are far less striking than in peptone shock. The character of the curve is different from that obtained in peptone shock. In experimental fat embolism, the rise induced by dyspnea is usually less marked, the ascent is more gradual, and the decline usually slower. The pressure tends to fall progressively lower between the periods of dyspnea, so that the tendency to recover is lacking. All the animals of this series whose blood pressures showed a permanent fall invariably succumbed. In several instances dyspnea appeared to hasten the end by causing acute pulmonary edema. The increased negative intraalveolar pressure as a result of the deeper and more forcible inspirations may have been a factor in the production of this edema as in the case of adrenalin pulmonary edema in rabbits described by Auer and Gates.[30]

Artificial respiration with a bellows has been found to cause a rise in pressure in experimental fat embolism in some dogs. This rise, when it does occur, is very gradual (Figs. 7 and 8).

In view of the marked differences in the state of the circulation in these two conditions, to explain the rise in arterial pressure accompanying or following dyspnea in fat embolism upon the same basis as the similar phenomenon in peptone shock, namely as a result of respiratory suction, as is done by Porter,[29] would hardly seem justified. Conditions are certainly not favorable in fatembolism for the activity of this force. (1) There in no convenient large reservoir of stagnating blood upon which respiratory suction can be exerted. Instead, there is a general venous stasis. The distribution of the blood in the body can be influenced by gravity. Thus, if the foot of the operating board is raised, the venous sinuses of the brain will be found at autopsy greatly distended with blood. If at autopsy the skull is opened before the thorax is disturbed, enormous quantities of blood flow from the opened sinuses, indicating the extreme degree of venous stasis. This does not occur in anaphylactic and peptone shock. (2) The right side of the heart is already overfilled with blood. To add more blood would only increase its burden and augment the tendency, already present, to acute dilatation. (3) A large portion of the vascular bed of the lungs is occluded. Any additional blood brought to the

[30] Auer, J., and Gates, F. L., *J. Exp. Med.*, 1917, xxvi, 201.

heart by respiratory suction or any other force could not be delivered to the left ventricle where it is needed to raise arterial pressure. (4) The venous pressure rises when the arterial pressure falls (Figs. 2 and 3). (5) The vessels still retain their functional integrity as shown by the reaction to adrenalin (Fig. 8).

A more reasonable explanation, but one difficult to verify, is that in experimental fat embolism dyspnea, and perhaps artificial respiration with a bellows, in some way facilitates the passage of blood through the lungs. Cloetta[31] has called attention to the effect of inflation of the alveoli upon the caliber of the interalveolar capillaries. When the lung is collapsed, the capillaries are reduced in diameter. With moderate inflation there is radial traction upon these vessels and their lumina are increased in size. With marked inflation, the capillaries are narrowed, both by compression and by linear extension. In dyspnea this cycle is repeated in rapid succession, so that a milking action upon the capillaries is produced which would tend to dislodge mechanically the occluding droplets of oil from these vessels. But the question of the mechanism by which dyspnea and artificial respiration cause a rise in blood pressure in experimental fat embolism must remain for the present without a satisfactory answer.

We may summarize the effects of dyspnea upon the low blood pressures in peptone and anaphylactic shock and in experimental fat embolism as follows: In the former, the rise in pressure is relatively sharp; there is a tendency to decline, but the pressure does not usually reach its former low level unless the dose of peptone was exceptionally large; and the animal generally recovers if the periods of dyspnea are repeated with sufficient frequency to prevent serious damage to the vital centers of the brain by the anemia. In experimental fat embolism, the rise in pressure accompanying dyspnea and the subsequent decline are usually more gradual than in peptone shock; the tendency is for the pressure to sink progressively lower, after each paroxysm of dyspnea; and no permanent benefit has been observed from the employment of dyspnea after the arterial pressure has once been materially and permanently reduced. If permanent

[31] Cloetta, M., *Arch. exp. Path. u. Pharm.*, 1912, lxx, 407.

improvement in surgical shock is found to follow repeated periods of dyspnea, this would appear to be indirect evidence that this condition is not due to fat embolism.

Further Differences between the Low Blood Pressure Associated with Peptone Shock and Experimental Fat Embolism.

In peptone and anaphylactic shock the respiration is usually not affected except for the temporary dyspnea that occasionally occurs during the fall in pressure. In experimental fat embolism, on the other hand, two very unlike changes in respiration have been observed in different animals. Sometimes both conditions have developed in the same animal at different stages of the experiment. In a number of dogs a violent dyspnea occurred, lasted for several minutes, and usually resulted fatally. This was almost always accompanied by edema of the lungs. The condition closely simulated the clinical picture of fat embolism sometimes seen after fractures of long bones. In several instances the difficulty of respiration became so extreme that it was necessary to disconnect the tracheal cannula from the ether bottle. With each violent expiration a shower of frothy fluid was blown from the cannula. The degree of edema appeared to bear a direct relation to the severity of the dyspnea.

In other animals a condition of apnea not infrequently developed unexpectedly without any change in the amount of anesthetic being given. This may occur before the critical quantity of oil has been reached in the injections, and therefore before the arterial pressure has begun to fall. The onset of apnea may be sudden, but usually it is somewhat gradual. The respirations become more shallow and less frequent until they stop entirely, as in fatal ether poisoning. If the anesthetic is removed promptly and moderate artificial respiration instituted at once, the animal can usually be revived, and after a varying period of time will begin to breathe spontaneously again. If the artificial respiration is not started without delay, the blood pressure quickly falls and the heart stops beating within a few minutes. A delay of $\frac{1}{2}$ minute has appeared, in some instances, to result in the death of an animal that might have been revived. This apnea has not been observed in peptone shock.

The similarity of this series of events to ether poisoning, and the recovery of the animal upon the removal of the anesthetic and the institution of artificial respiration, has led to the tentative explanation that the toxicity of ether may be enhanced in experimental fat embolism. The recovery of the dog under the conditions noted would seem to exclude the possibility of any organic damage to the respiratory center by the lodgment therein of an embolus of oil. Careful postmortem examination, gross and microscopic, of the region of the floor of the fourth ventricle has not revealed evidence of such a lesion.

Janeway and Jackson[3] reduced arterial pressure by compressing the inferior vena cava. Release of the compression after 2 hours was followed by a prompt rise in arterial pressure, which slowly fell again, and the animal died in about 12 hours. They consider that the dilatation of the peripheral venules and capillaries as a result of the increased venous pressure caused such a loss of tone that even after normal pressure was restored they became overfilled with blood. Too little blood was thus permitted to reach the heart to keep the arterial pressure above the danger zone of 40 to 50 mm. of mercury, and the animal died.

The animals used in this series of experiments were observed for varying periods of time after the injection of oil. In most instances the experiments did not last longer than 5 hours. Hence from the observations of Janeway and Jackson, it might be objected that an amount of fat in the lungs less than the critical quantity may cause a fall in pressure more than 5 hours after the injection. That there is a rise in venous pressure in experimental fat embolism is known from the work of Warthin,[4] and of Bissell,[5] and from the results here reported. But as far as these experiments go, it would seem that a rise in venous pressure of a lasting character or of a degree greater than the fluctuations observed in dogs without any further treatment than the administration of an anesthetic, does not begin until the critical quantity of oil has been injected and the arterial pressure has begun to fall. Even then the venous pressure (external jugular-superior vena cava) may be lowered by dyspnea. The conditions present in experimental fat embolism do not, therefore, appear to be analogous to those in the experiments of Janeway and Jackson until a relatively large amount of oil has been administered. After

this point has been reached, that is after the venous pressure has begun to rise, progress to a fatal termination is usually rapid. Hence there is no reason to consider this possible objection valid.

SUMMARY.

1. In peptone shock there is a marked, precipitate fall in arterial pressure. At the same time there is a fall in venous pressure.

2. In experimental fat embolism, (*a*) the fall in blood pressure is always gradual; (*b*) approximately 1 cc. of oil for each pound of body weight must be injected before a lasting fall in arterial pressure is produced; (*c*) it makes only a slight difference whether this amount is injected in small doses at a time or in relatively large quantities; and (*d*) when the arterial pressure falls, but not till then, the venous pressure rises.

3. In peptone shock, dyspnea, by its suction and force-pump action upon the reservoir of stagnating blood in the liver, brings more blood to the heart and causes a rise in arterial pressure. By repeatedly inducing short periods of dyspnea at frequent intervals, permanently beneficial results are obtained and the life of the animal can be saved.

4. In experimental fat embolism, dyspnea will cause a rise in blood pressure. But permanently beneficial results have not been obtained by this method. If dyspnea is found to bring permanent improvement in surgical shock, it is indirect evidence that this condition is not due to fat embolism. Respiratory suction is probably not responsible for the rise in blood pressure in experimental fat embolism. It seems more likely that the dyspnea in some way facilitates the passage of blood through the embarrassed pulmonary circulation. Artificial respiration with a bellows will also frequently cause a rise in blood pressure in experimental fat embolism.

5. In peptone shock the respiration is usually not affected, although there is some evidence that the respiratory center may be in a state of increased irritability. In experimental fat embolism, in some animals a violent dyspnea develops spontaneously. This is usually accompanied by edema of the lungs. In other instances, an apnea occurs, even before the blood pressure has begun to decline.

EXPLANATION OF PLATES.

Plate 17.

Fig. 1. The fall in arterial and venous pressures associated with peptone shock. Dog 3; weight 42 pounds. The writing point of the venous pressure manometer was recording $\frac{3}{4}$ inch in advance of that of the arterial manometer.

Fig. 2. The effect on arterial and venous pressures of injecting small doses of oil at frequent intervals. This animal had been previously subjected to peptone shock, which accounts for the gradual rise in arterial pressure in the first section of the tracing (*a*), in spite of the injections of oil. Between the first and second (*b*) sections of the tracing, 4 minutes elapsed and 6 cc. of oil were injected. After the arterial pressure had begun to fall, the respiration became slow and four inspirations with the bellows were used as shown in the second section of the tracing. Dog 11; weight 15 pounds.

Plate 18.

Fig. 3. The effect on arterial and venous pressures of the injection of small doses of oil at frequent intervals. The same animal as in Fig. 1. Between the first (*a*) and second (*b*) sections of the tracing 20 minutes elapsed. During this time a clot formed in the venous cannula. In removing this clot some fluid was lost from the system of transmission. This probably accounts for the lower level of the venous pressure tracing in this section of the figure. In the second section the arrows indicate corresponding positions of the writing points of the two manometers.

Plate 19.

Fig. 4. Dog 14. The effect of nicotine on arterial pressure in peptone shock with and without the production of dyspnea. *a* and *b*, injections of adrenalin and nicotine, respectively, before the induction of shock; *c*, peptone injected; *d*, adrenalin; *e*, nicotine without dyspnea; *f*, nicotine with marked dyspnea. Dosage 1 cc. of 1: 50,000 adrenalin, and 1 cc. of 1: 4,000 nicotine.

Fig. 5. The effect of rapidly injecting large single doses of oil at frequent intervals, the total amount being less than the critical quantity. Moderately rapid fall in pressure with gradual rise to previous level. Dog 23; weight 30 pounds. The time covered by the tracing, including the period during which the drum was stationary, was 18 minutes.

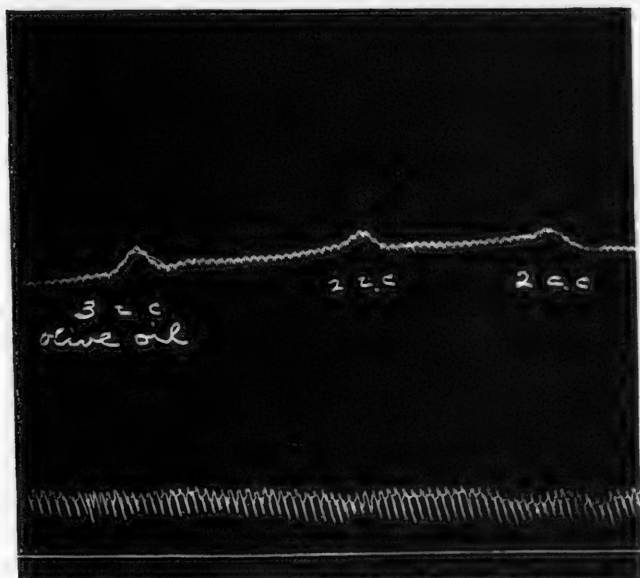
Plate 20.

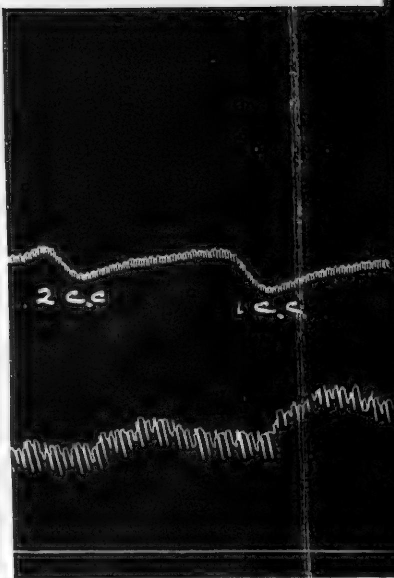
Fig. 6. Gradual fall in arterial pressure after rapid injections of large amounts of olive oil (75 cc.). The effect on respiration is also shown. Female dog; weight 29 pounds. Time covered by entire tracing, 7 minutes.

FIG. 7. The effect of artificial respiration with a bellows upon low blood pressure (arterial) in experimental fat embolism. The animal was breathing spontaneously when the artificial respiration was instituted. Time covered by tracing, 4 minutes.

PLATE 21.

FIG. 8. The effect of artificial respiration with a bellows upon low arterial pressure in experimental fat embolism. The respiration became very slow and finally stopped. Rapid fall in pressure, followed by gradual rise during artificial respiration. Between the first (*a*) and second (*b*) sections of the tracing $3\frac{1}{2}$ minutes elapsed. During this time the pressure continued to rise gradually. Respiration became spontaneous while the drum was stopped. The rise in pressure indicated by the perpendicular line occurred very quickly upon the suspension of artificial respiration before the drum could be started. This tracing also shows the reaction to adrenalin in the low blood pressure due to experimental fat embolism in contrast to the absence of this reaction in peptone shock.





a

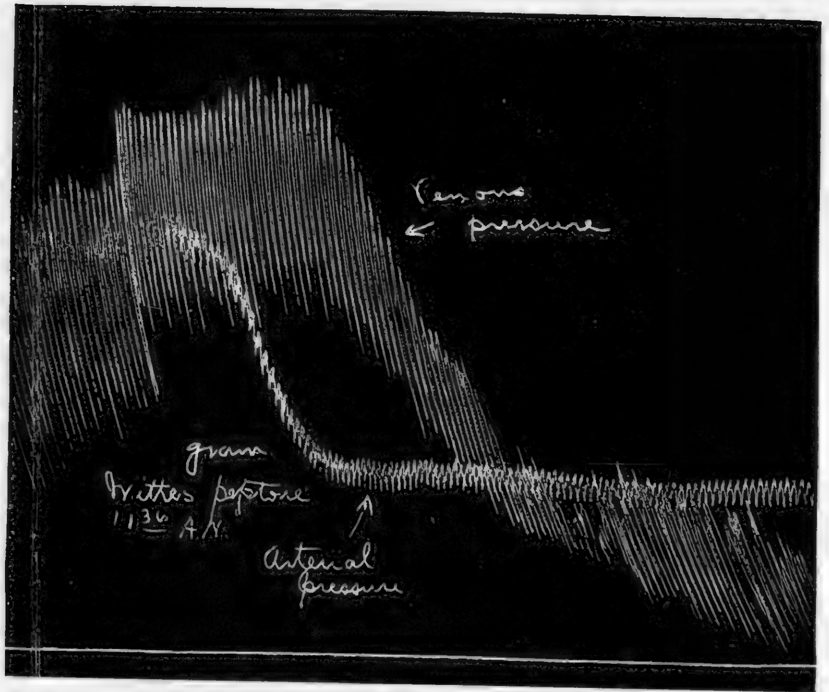
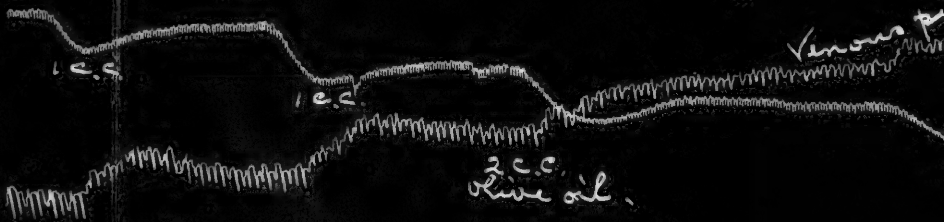
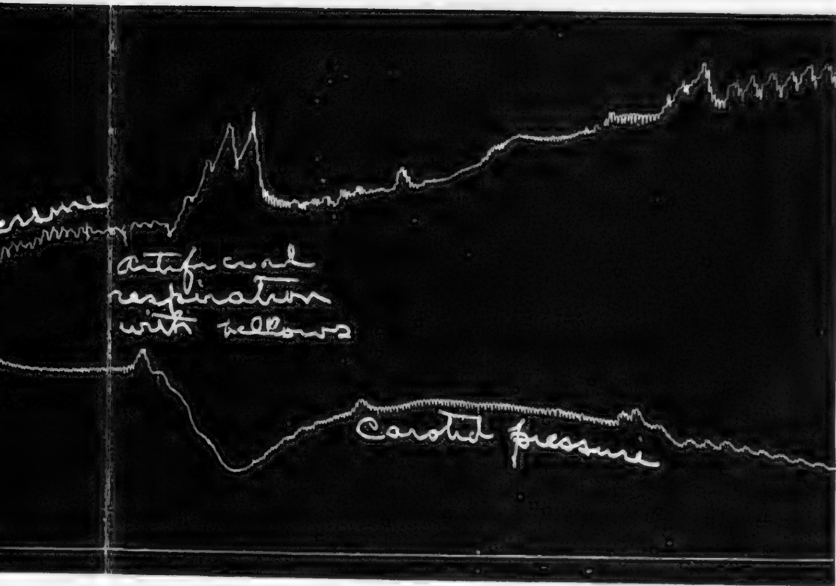


FIG. 1.

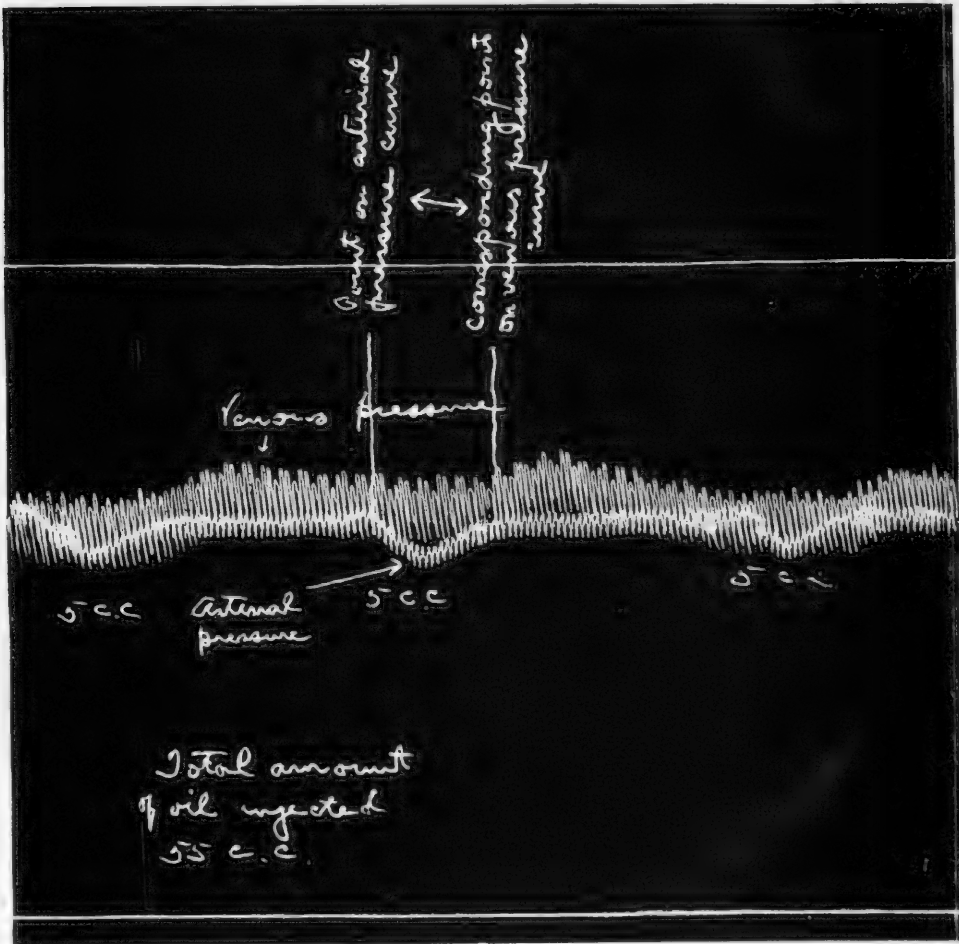


b

FIG. 2.



(Simonds: Low blood pressures.)



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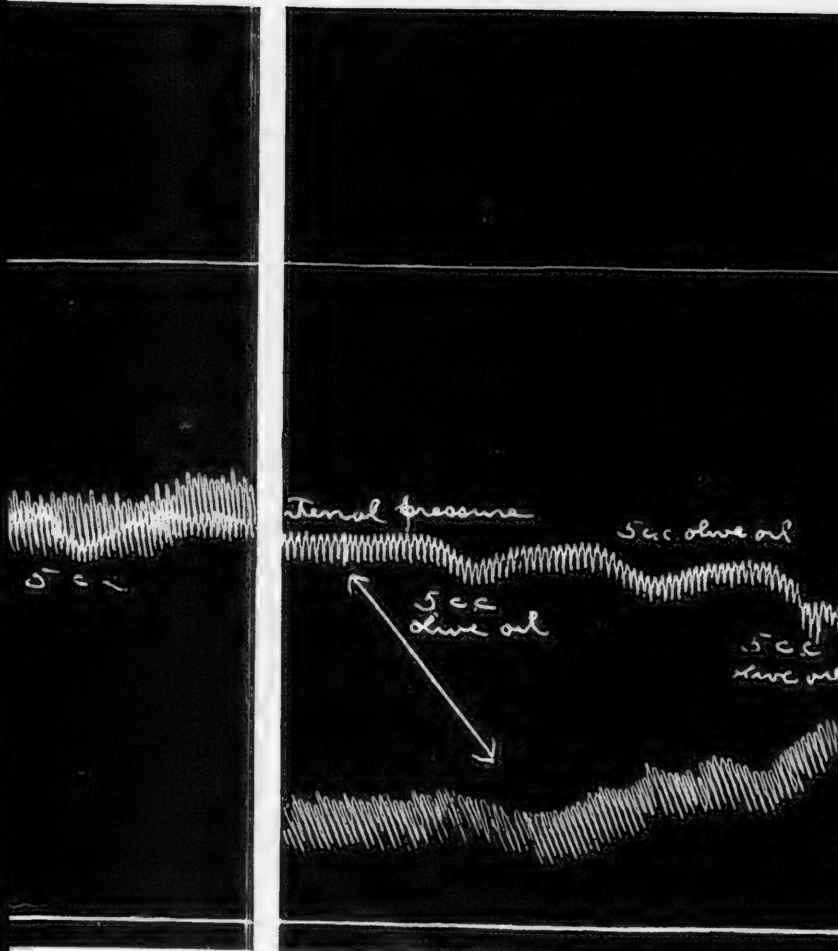
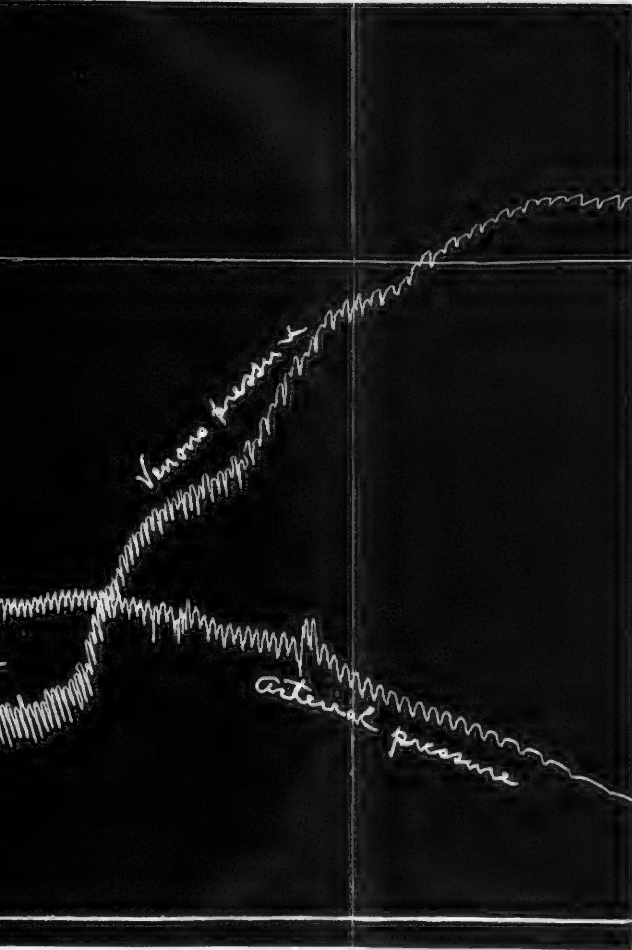


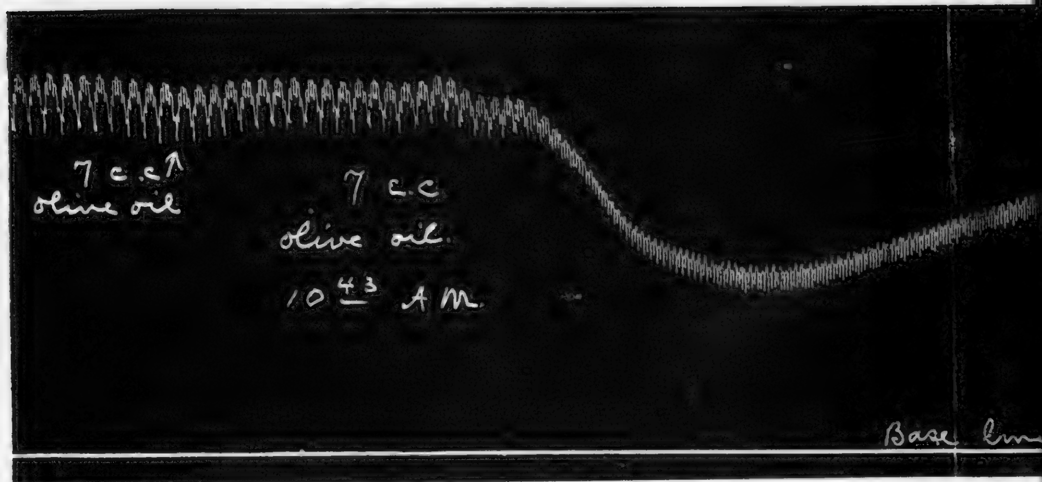
Fig. 3.

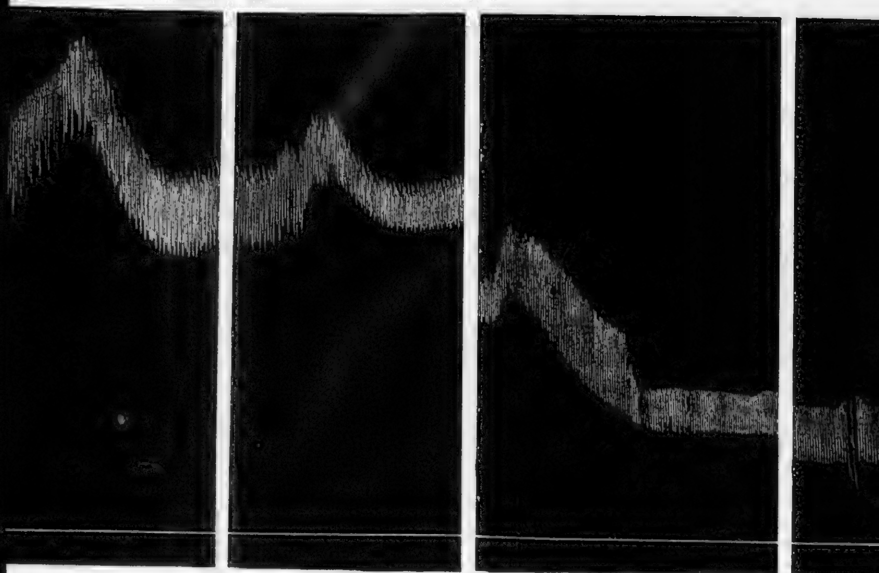


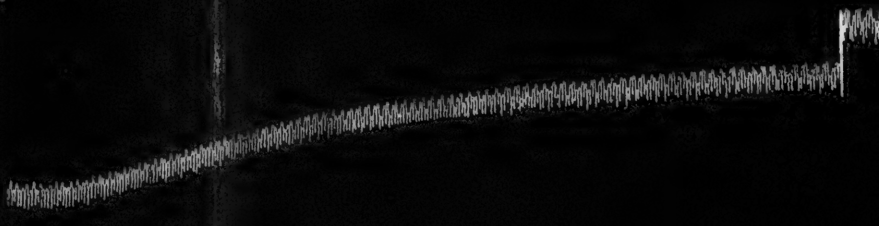
b

(Simonds: Low blood pressures.)

a




a
b
c
d


Base line - arterial pressure

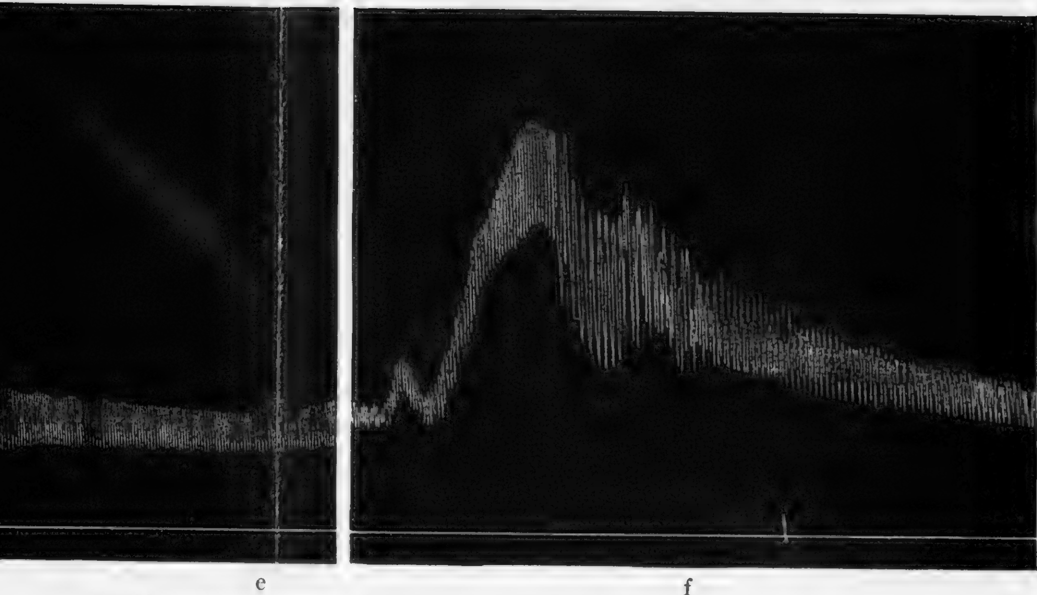


FIG. 4.

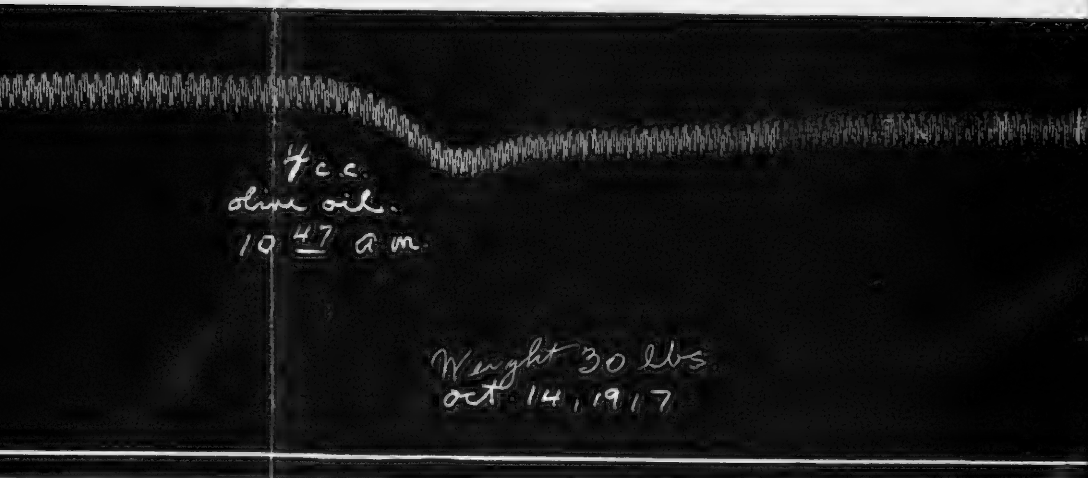
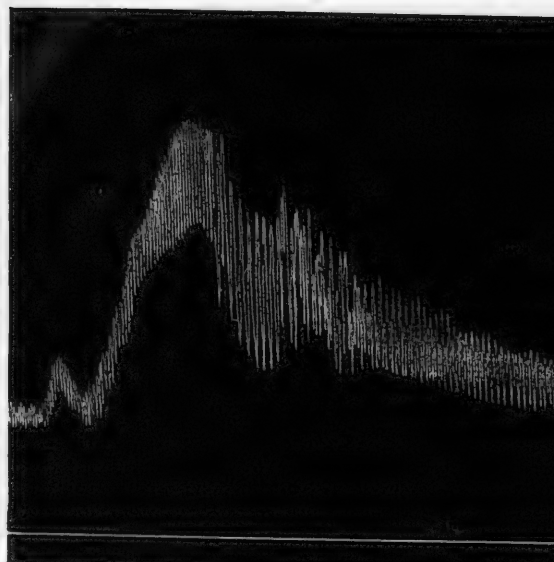


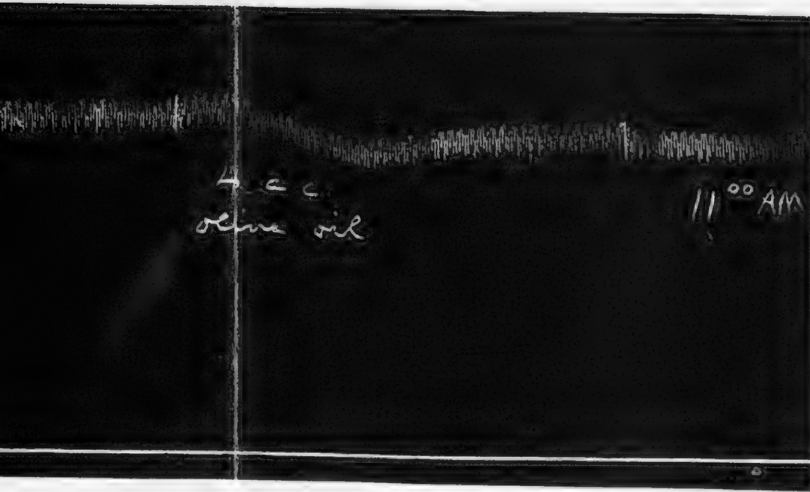
FIG. 5.

e



f

PLATE 19.



(Simonds: Low blood pressures.)

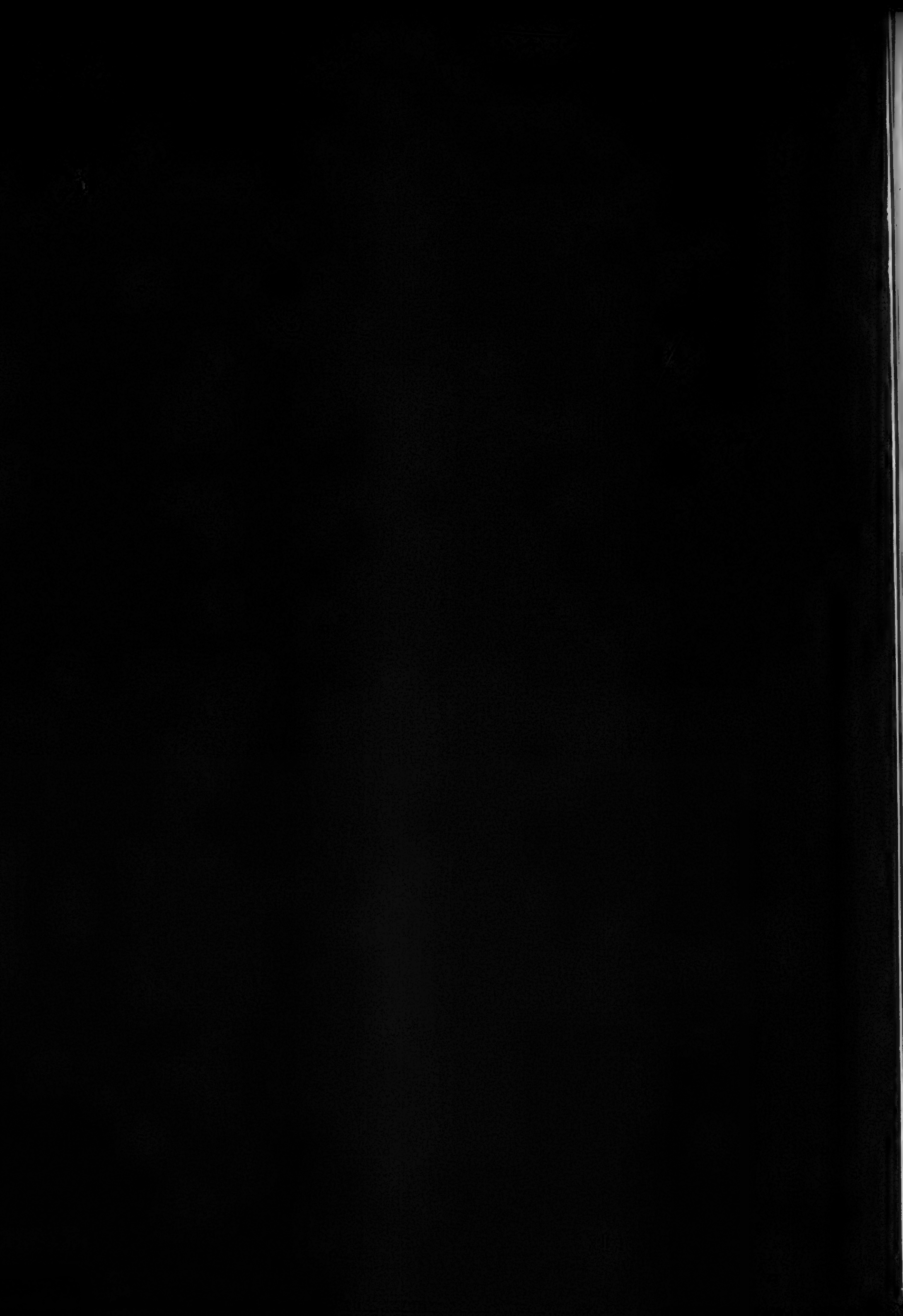


FIG. 6.

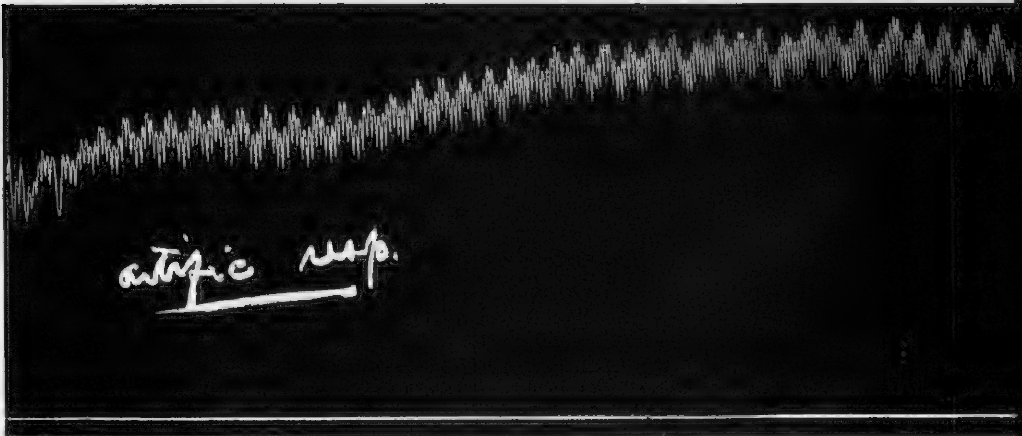
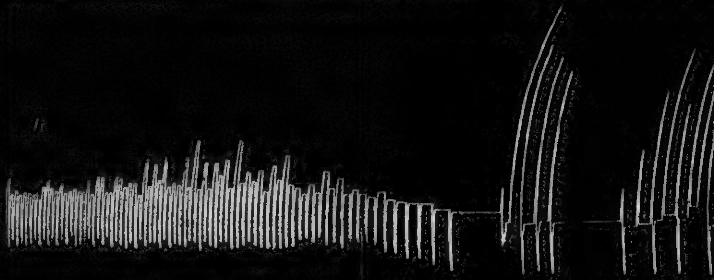


FIG. 7.



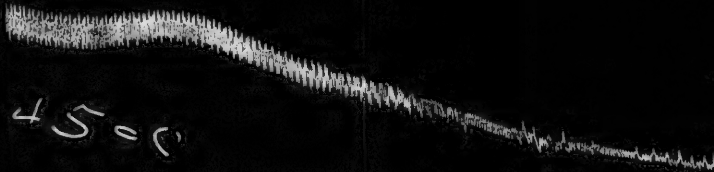


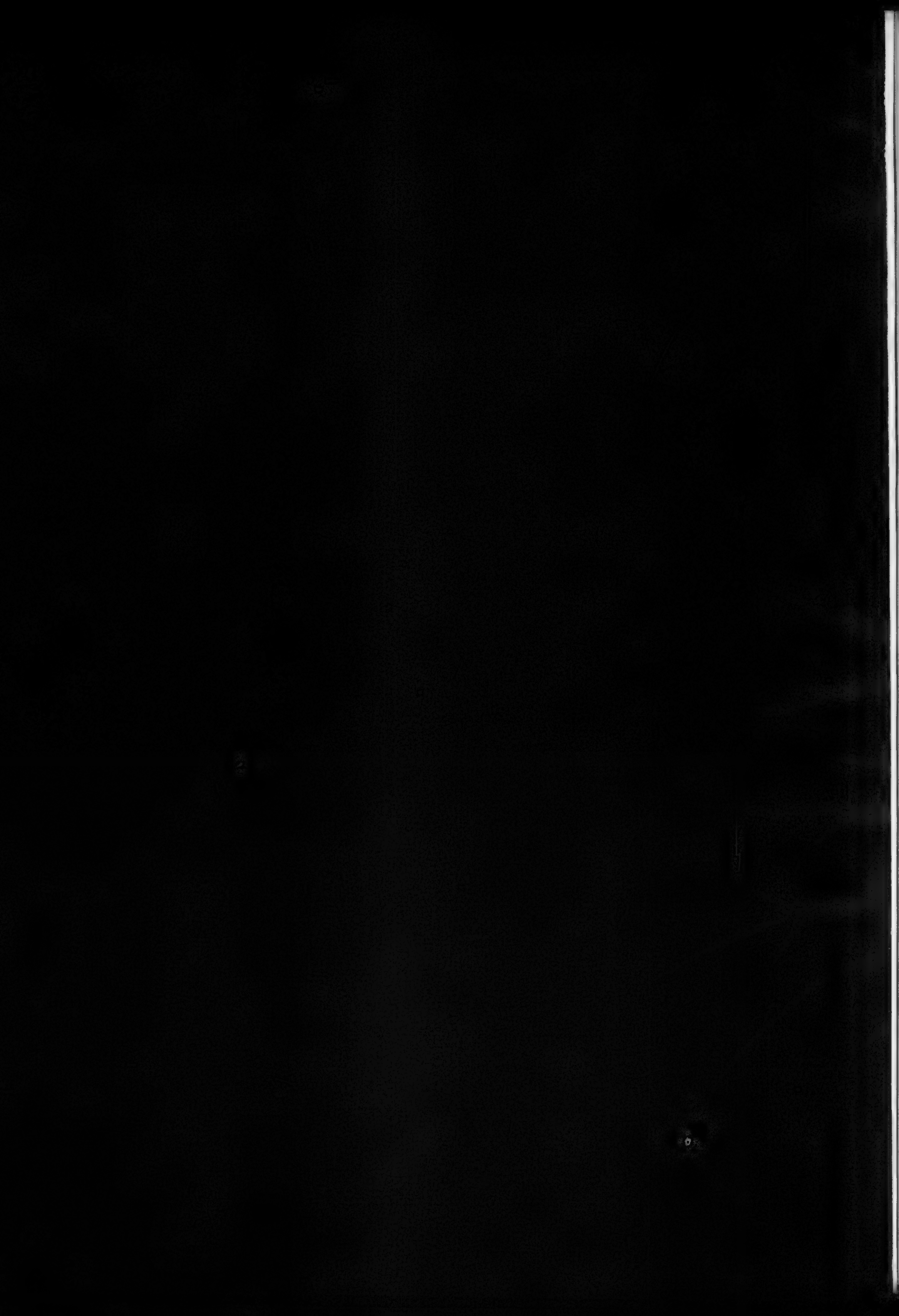
Fig. 6.

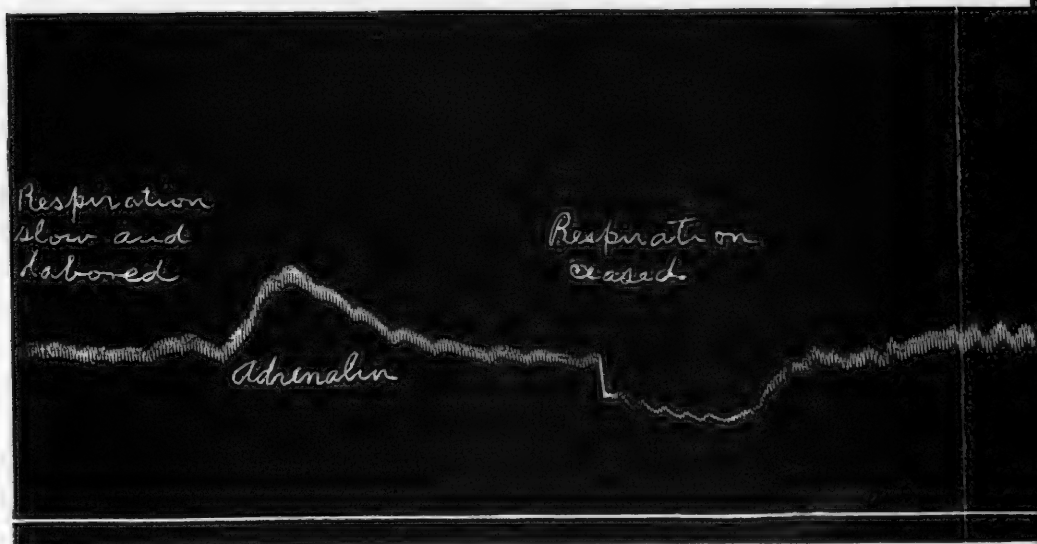
Fig. 7.

(Simonds: Lo

PLATE 20.

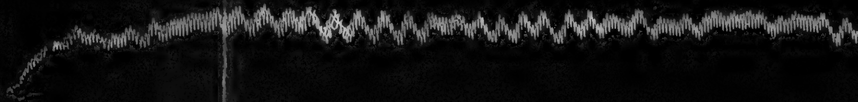
w blood pressure.)





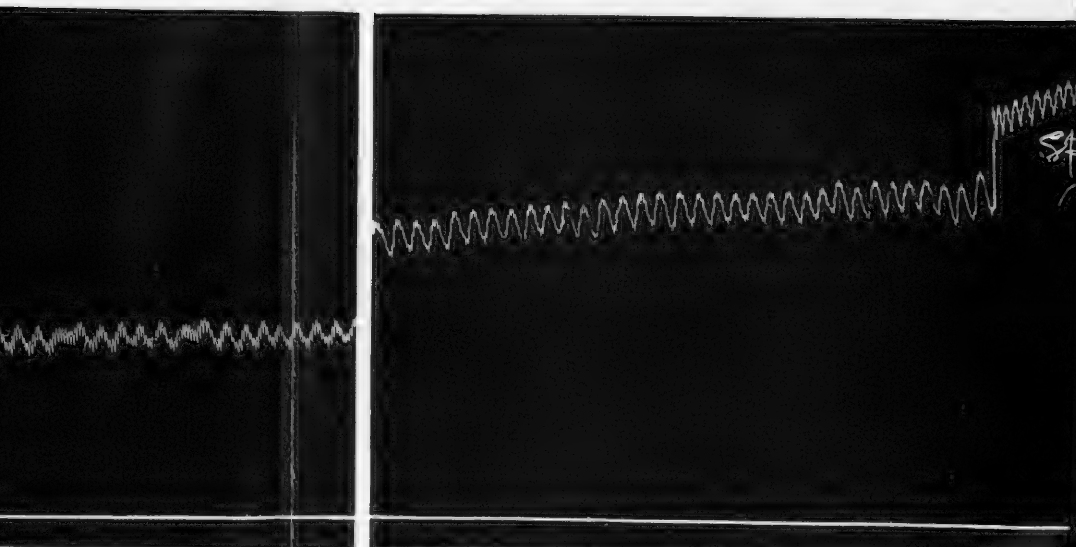
a

Artificial Respiration



a

Fig. 8.



b

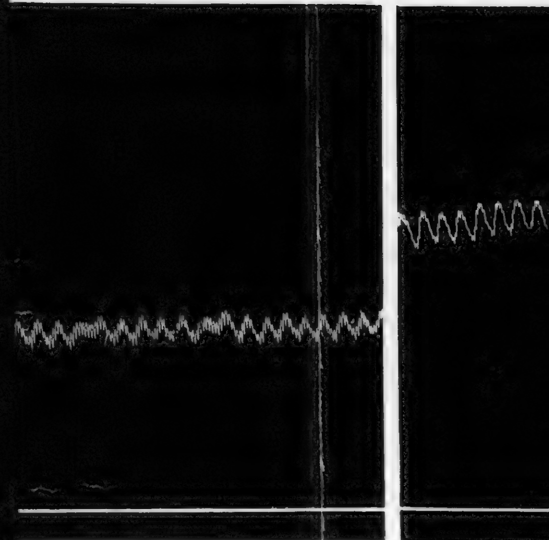
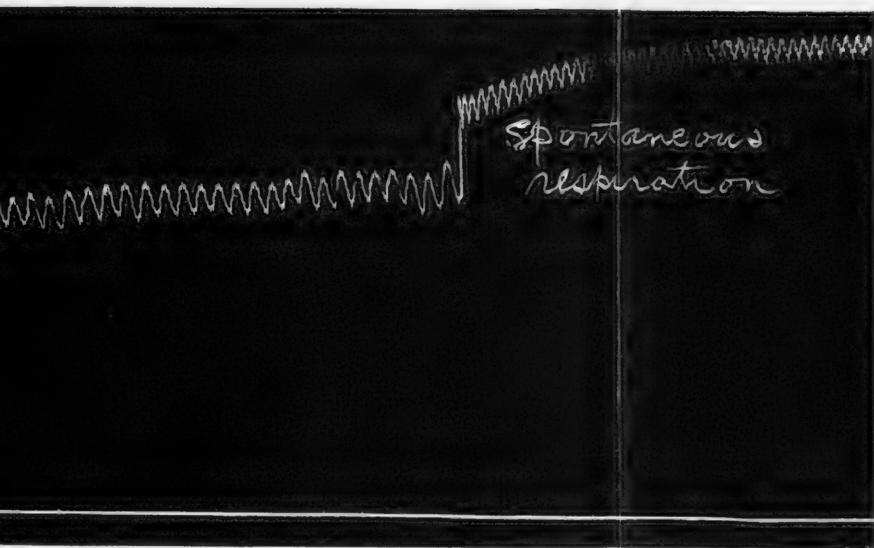
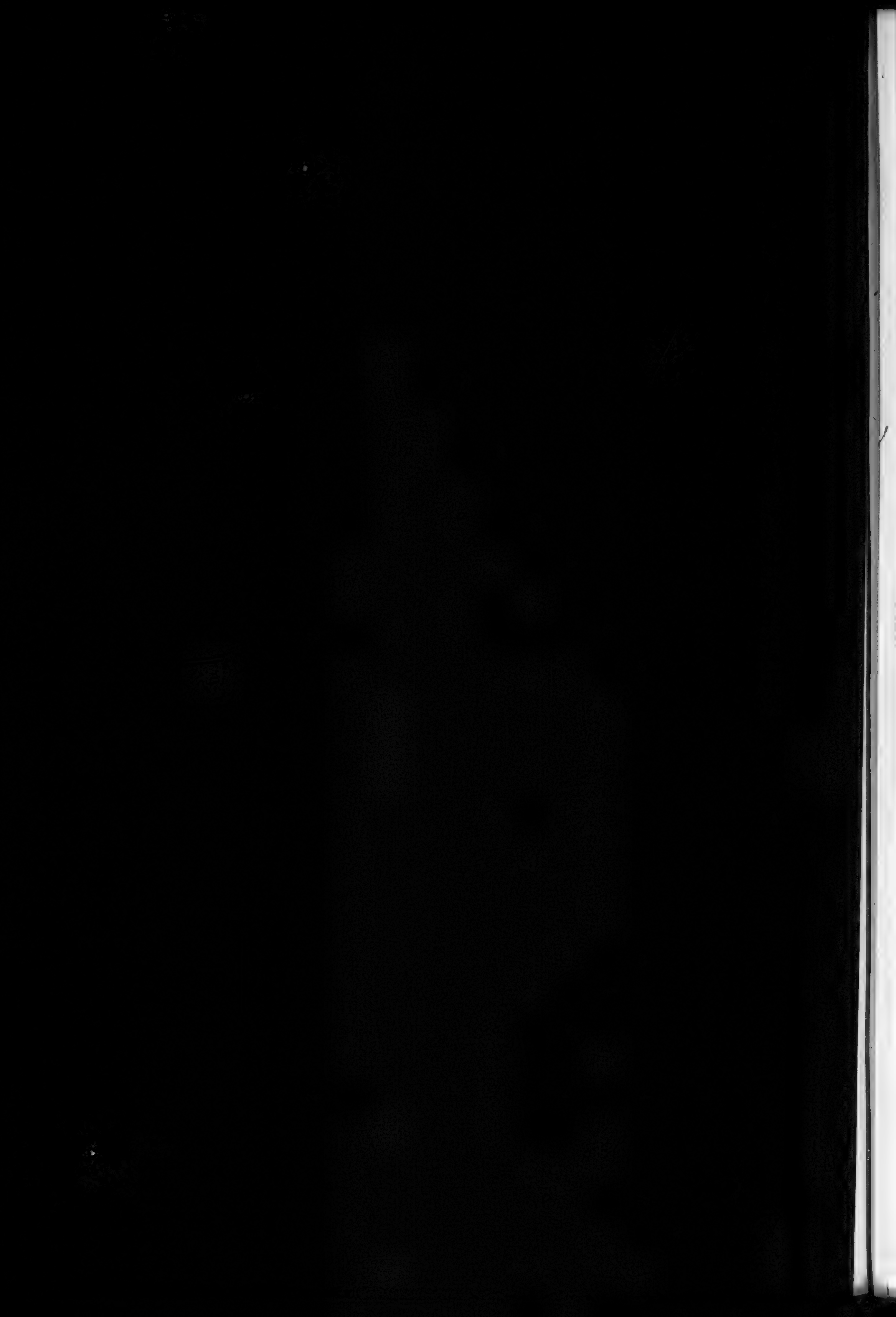


PLATE 21.



b

(Simonds: Low blood pressures.)



AUTOHEMAGGLUTINATION EXPERIMENTALLY INDUCED BY THE REPEATED WITHDRAWAL OF BLOOD.

By OSWALD H. ROBERTSON, M.D., and PEYTON ROUS, M.D.

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PLATE 22.

(Received for publication, March 11, 1918.)

There is much evidence in the literature to show that moderate losses of blood act to increase the titer of antibodies developed in response to an injected antigen.[1] Little attention, though, has been given to whether the production of antibodies normal to the organism is likewise stimulated. The point has considerable clinical interest, especially in connection with the remarkable resistance to infection manifested by many patients with severe anemia. In the work now reported one phase of the problem has been taken up; namely, the influence of repeated bleedings on the normal isohemagglutinins.

Experiment 1.—Interagglutination tests were carried out with the cells and sera of twelve normal rabbits. The cells had been twice washed and made to 5 per cent suspensions with salt solution. The sera were obtained from the clot at room temperature (22°C). Mixtures of each serum with each cell suspension in equal parts were kept at 22°C. for 15 minutes and examined with the microscope. Agglutination was found in a moderate proportion of the 144 mixtures. Autoagglutination was not noted.

On the basis of the findings, the animals were separated into two groups, each possessing about the same proportion of material for isoagglutination; that is to say, susceptible cells and agglutinating sera. One group of six rabbits was set aside as controls, and the remaining six rabbits were bled 10 cc. from the heart every 3 to 6 days during a period of 2 months. All were kept under the same conditions. From time to time the interagglutination tests were repeated, sometimes by the method just described, sometimes by mixing the whole citrated bloods in the proportions of 9 to 1 and 1 to 9, according to the method of Rous and Turner.[2]

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[1] See Lippmann, *Z. exp. Path. u. Therap.*, 1914, xvi, 124, for large bibliography.

[2] Rous, P., and Turner, J. R., *J. Am. Med. Assn.*, 1915, lxiv, 1980.

Experiment 2.—Fourteen rabbits were used in two groups arranged on the same basis as in Experiment 1, but with only six individuals in the control group. The other eight animals were bled as already described during a period of 26 days. Interagglutination tests were carried out between the control animals and between these and the bled animals, but not between the individuals of this latter sort. Citrated bloods were used in the tests, which were repeated at intervals of a week or more. Tests with sera and washed cells were also made when this seemed advisable. In whole citrated bloods autoagglutination is easily seen. None was discoverable prior to the bleedings.

No Induced Isoagglutinins.

A number of the rabbits possessed isoagglutinins to start with. Some were bled, and some kept as controls. The bleedings had no demonstrable effect to alter the isoantibodies or to cause an appearance of new ones. It is true that weak isoagglutinins sometimes developed in individuals possessing none to begin with, but they were found to practically an equal degree in the controls and were probably in the nature of intercurrent serum changes such as Ottenberg and Thalhimer[3] have reported.

Clumping in the Shed Blood.

In five out of fourteen of the bled rabbits there developed a tendency of the red cells to clump together into masses in the shed blood. The clumping was never general, bringing together all the cells, as in the case of rabbits repeatedly transfused,[4] but the cell masses lay scattered here and there amid free cells. The phenomenon was not found in any of the twelve controls, nor has it since been observed in a large series of other normals. In instances of anemia, on the other hand, resulting from malnutrition, it has sometimes been met with.

The clumping phenomenon was definitely associated with the anemia following in some instances on the bleedings. Many medium sized rabbits withstand excellently the loss of 10 cc. of blood every 3 to 5 days during a long period. Their hemoglobin percentage and the appearance of the corpuscles remain practically unaffected. The clumping was never noted in these animals. In other rabbits the

[3] Ottenberg, R., and Thalhimer, W., *J. Med. Research*, 1915–16, xxxiii, 213.
[4] Rous, P., and Robertson, O. H., *J. Exp. Med.*, 1918, xxvii, 509.

bleedings rapidly brought about a moderate anemia with pale corpuscles, microcytes, and poikilocytes in circulation. In such instances the clumping occurred, though it was by no means a regular accompaniment of the condition. The number of bleedings and total loss of blood prior to appearance of the clumping were sometimes surprisingly small. Clumping was well marked in a 1,500 gm. rabbit 3 days after the last of two bleedings of 10 cc. each with an interval of 3 days between. In another animal of 1,350 gm., also bled twice, but at an interval of 7 days, the phenomenon was visible 3 days after the last bleeding. In both cases the blood loss was very poorly borne.

The clumping was plainly apparent in whole citrated bloods[5] allowed to stand for 15 minutes or more at room temperature and examined microscopically after dilution with salt solution. When at all marked it could be seen in thick slide preparations of the blood, as such, providing the cells were not numerous enough to interfere with the observations (Fig. 1). Under these circumstances it appeared within about a minute after the blood was shed and before any clotting had occurred. Each clump consisted of from 3 or 4 to 40 or 50 corpuscles massed helter-skelter.

Cause of the Clumping.

The question whether the phenomenon had its cause in a change in the cells, or plasma, or in both, was largely answered by the routine tests of Experiments 1 and 2. These demonstrated that the cells of the bled rabbits had undergone no alteration as regards agglutinability or inagglutinability by normal sera of known behavior. Furthermore, the clumping, like that due to the autoagglutinin present in normal plasma[6] and the principle present in the plasma of transfused rabbits,[4] did not occur at body heat. The cells remained free in citrated blood at 38°C., and the massing together which was visible at room temperature disappeared when the specimen was warmed. Corpuscles separated from the citrated plasma while warm, then washed in warm salt solution and brought together in it at room temperature,

[5] 10 parts of blood to 1 part of a watery 10 per cent solution of sodium citrate.
[6] Landsteiner, K., *Münch. med. Woch.*, 1903, i, 1812.

failed entirely to clump. But when a little of the thick cell suspension was dropped into the original citrated plasma, the cells massed together at once. All these facts showed that the clumping was due to an element in the serum and that this element has much in common with the normal and induced autoagglutinins.

Distinguishing Traits of the Agglutinin.

The agglutinin of the bled rabbits was able to cause clumping in the whole blood as such, or in the whole citrated blood, at room temperature (22°C.), whereas the normal agglutinin is effective at 22° only when a large amount of serum is allowed to act on a few cells.[5] The agglutinating principle of the bled rabbits, obtained in the free state, as in serum separated from the cells by defibrination and centrifugation at 38°C., was so strong in all cases as to bring about clumping at 22°C. in mixtures of the serum with an equal part of a 5 per cent suspension of the animal's own washed cells, and in some instances when there was a further dilution with one part of salt solution. The serum of five normal rabbits similarly separated and tested yielded not the least trace of agglutination. These results with a constant amount of antigen (the 5 per cent cell suspension) rule out the possibility that clumping in the anemic rabbits was due merely to the action of the normal autoagglutinin on an antigen (the red corpuscles) diminished in quantity by the bleedings.

Attempts to obtain the agglutinating factor in salt solution led to a singular finding. Normal autoagglutinins become much more effective as cooling proceeds from room temperature to 0°C. and are best demonstrated in the cold. The agglutinin of the bled rabbits, on the other hand, while effective at room temperature, may be relatively little enhanced in activity by further cooling, and at 0°C. may be surpassed in its action by the normal antibody.

Experiment 3.—A few cubic centimeters of blood were obtained from each of two normal rabbits and two which had been repeatedly bled and were the possessors of an agglutinin which caused clumping at room temperature. The sera were separated from the cells by defibrination in the warm, and centrifugation in a water jacket at 38°C. Those of the bled animals caused clumping at room temperature when mixed with an equal volume of a 5 per cent suspension of the corresponding cells twice washed in the warm. In similar mixtures of normal

sera no clumping occurred. Now 1.2 cc. of each serum was mixed with 0.1 cc. of a 50 per cent suspension of the corresponding cells, and the tubes were plunged in melting ice. The results are given in Table I.

It will be observed that the agglutinin of the normal rabbit No. 2, while practically ineffective at room temperature (22°C.), caused a more complete clumping at 0°C. than did the agglutinins of the bled rabbits, which were so active at 22°C. A second experiment along these lines gave similar results. The data do not enable one to say whether two distinct sets of antibodies are here concerned, but they

TABLE I.

Rabbit.	Room temperature (22°C.). Microscopic observations after 15 min.				0°C. Macroscopic observations. 1.2 cc. serum + 0.1 cc. 50 per cent red cells.	
	Whole blood.	3 parts serum + 1 part 5 per cent red cells.	1 part serum + 1 part 5 per cent red cells.	1 part serum + 1 part salt solution + 1 part 5 per cent red cells.	After 8 min.	After 66 min.
Normal.						
1	0	0	0		+	Sedimentation incomplete; sediment finely granular.
2	0	Tr.	0		++++	Complete sedimentation into a single, solid mass.
Bled.						
3	+−	+	Tr.		+++	Sedimented completely into fairly large masses.
4	+		+	Tr.	++++	Complete sedimentation into a few large masses.

show clearly that the effects of an autohemagglutinin at one temperature cannot safely be taken as the indicator of the effects at another. The study of such antibodies assumes in consequence great complexity.

Agglutination and Rouleau Formation Are Not Connected.

The observations of several authors have led them to conclude that rouleau formation is intimately connected in its cause with agglutination. Our findings in transfused rabbits[4] would seem also to point to this, since the appearance of new agglutinins in the blood is

always preceded by exaggerated rouleau formation. But the present results with rabbits repeatedly bled prove that the association is not obligatory. Here a partial or complete loss of the tendency to rouleau formation was regularly noted to accompany the development of the autoagglutinin (Fig. 1).

SUMMARY.

The repeated withdrawal of moderate quantities of blood does not lead to a development of new isoagglutinins in rabbits, or to noteworthy changes in normal ones already present. On the other hand, clumping of the animals' own cells in specimens of the whole blood is a frequent result. It is found in animals rendered anemic by the bleedings, not in those that rapidly repair their losses and remain in good condition. A similar clumping is sometimes to be seen in the blood of rabbits rendered anemic by malnutrition.

The clumping is due to a true autoagglutinin, which differs from the normal autoagglutinin in its far greater strength, as also, at least in some instances, by a peculiar variation in its activity with changes of temperature.

In the rabbits which developed isoagglutinins after bleeding, the tendency of the cells to form rouleaux was far less than the normal. It follows that rouleau formation is not essentially connected with autoagglutination, as has been assumed in the past.

In the light of the present findings a systematic search for autohemagglutinins in the blood of anemic patients would seem of interest. They have been noted in sick human beings (Ascoli, Klein), but not in recent years. The reason for this may well be that present day blood examinations are not of a sort to bring about their discovery. Thick films of fresh blood are seldom used for clinical purposes, and it is in thick preparations that clumping is most readily observed.

EXPLANATION OF PLATE 22.

Fig. 1. Autoagglutination in the blood of a rabbit rendered anemic by bleeding. Fresh slide-and-cover-glass preparation.

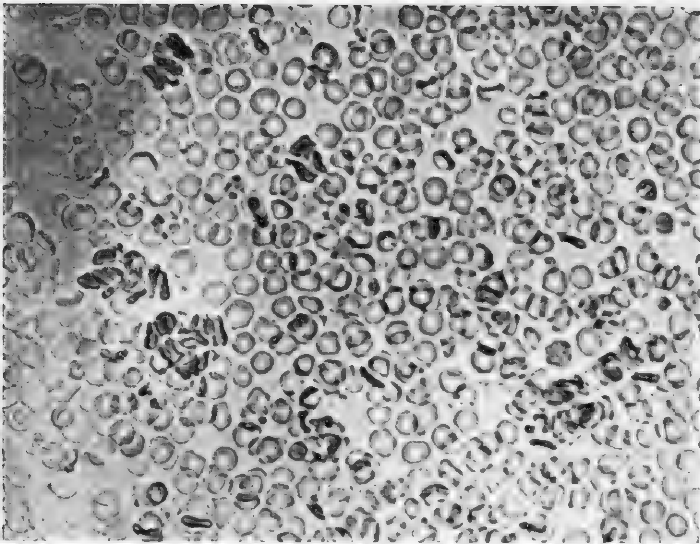
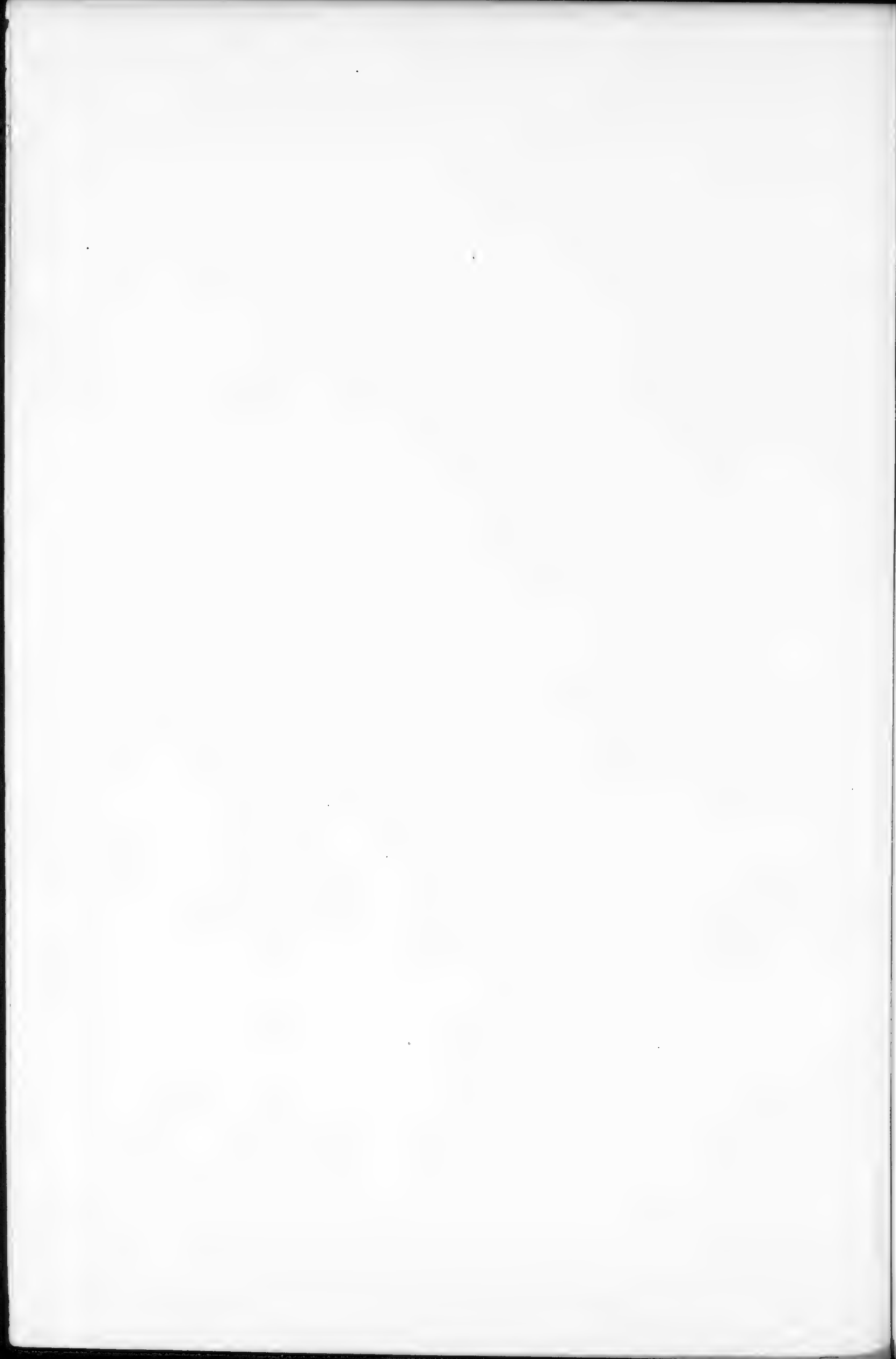


Fig. 1.



EXPERIMENTS OUTLINING THE LIMITATIONS OF OPERATIONS ON THE ABDOMINAL AORTA.

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Plates 23 and 24.

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From the results of the five experiments presented in this report, there seems to be no difficulty in correcting injuries of the abdominal aorta in dogs, with subsequent perfect restoration of the continuity of the vessel. The complete occlusion of the aorta for a period of 30 minutes is not necessarily followed by serious consequences.[1] For lateral defects in the aorta, or for injuries not involving the entire circumference, a rectangular clamp may be applied for a prolonged period in order that the circulation should not be completely cut off. An operative field free from blood can thus be obtained while the circulation is maintained through the remaining lumen of the aorta.

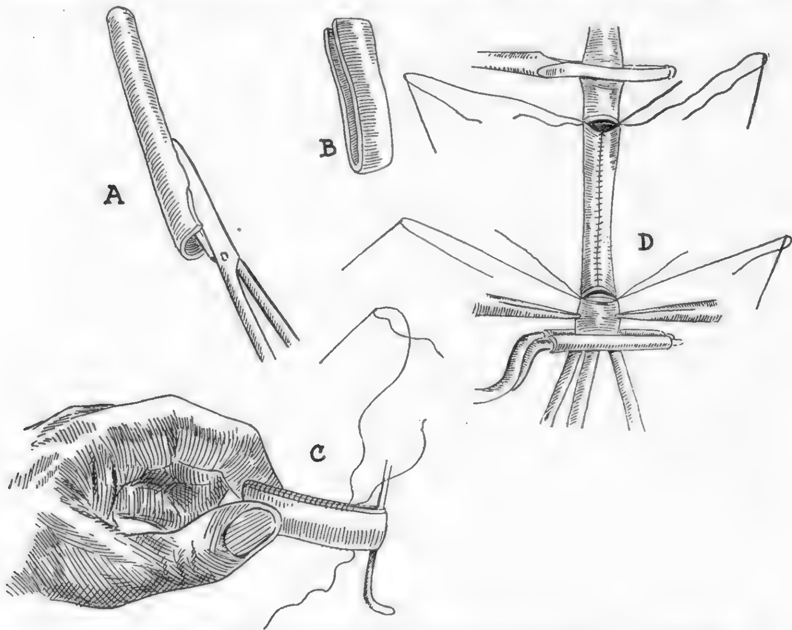
In the instances in which a portion of the aorta must be resected, an arterial segment taken from another animal can be safely utilized as a transplant. Smaller vessels can be adapted to the caliber of the aorta by the following procedure which was devised by Jeger and Helmuth Josef[2] (Text-fig. 1). The artery is split longitudinally (*A*), the ribbon thus formed is then folded double (*B*), and both edges are closed with a continuous suture of very fine silk (*C*). In this way, a tube, double the original size of the vessel is made, which is then transplanted end to end between the resected ends of the aorta (*D*).

Further experiments were undertaken to determine the practicability of reestablishing the continuity of the severed aorta by the cir-

[1] All operations were performed under complete ether anesthesia.

[2] Jeger, E., Die Chirurgie der Blutgefässe und des Herzens, Berlin, 1913.

cular suture. The results obtained seem to indicate that while the operation is possible, the difficulty of approximating the severed ends during the suture is greatly increased, on account of the retraction of the aortic tube. The approximation entails such injury that the probability of thrombosis is much increased. Therefore, when the aorta is completely severed, the introduction of a transplanted seg-



TEXT-FIG. 1. Method for transplanting an arterial segment to the aorta. *A*, first stage of the operation, *B*, second stage, *C*, third stage, and *D*, fourth stage.

ment would be indicated and the procedure would be the same as when a portion of the aorta had been removed.

Danis,[3] in an exhaustive study, showed that the ligature of the abdominal aorta in human beings has hitherto always resulted fatally. Braun[4] has reported a successful suture of the abdominal aorta on a 6½ year old girl with a ganglioneuroma about 4 pounds in weight.

[3] Danis, R., Anastomoses et ligatures vasculaires, Brussels, 1912.

[4] Braun, cited by Jeger,[2] p. 236.

EXPERIMENTS.

Suture of a Longitudinal Incision in the Aorta.

Dog 1.—March 29, 1916. The aorta was exposed below the renal vessels by a median laparotomy. An incision 2 cm. in length was made through all the coats of the right anterolateral wall of the aorta. A rectangular clamp was applied to the aorta in order to shut off the blood from the operative field without completely occluding the aortic circulation. The incision was then closed with a continuous suture of silk. The parietal peritoneum was sutured over the aorta, and the laparotomy wound was closed by suture in layers.

Autopsy.—The dog was killed June 5, with chloroform. There were no evidences of infection. The abdominal wound healed by primary intention. The peritoneum was free from adhesions. The peritoneum covering the aorta was perfectly smooth, and showed no signs of a recent operation.

Gross Examination.—A cross-section of the suture line shows that the vessel is not surrounded by adventitious tissue except at the site of the suture. At this point the vessel wall is but slightly thickened. The intima is relatively smooth except along the suture line. Here there are slight, flat elevations of coagulated blood. These coagulations are old; their surfaces are smooth and they project into the lumen but slightly. New accretions of coagulated blood are apparently taking place at the site of the old blood coagulations described above.

Microscopic Examination.—Section of the vessel wall shows that the structure is intact and the wall preserved. The intima is smooth at the point of this section and shows no plaques of fibrin.

Suture of a Lateral Defect in the Aorta.

Dog 2.—Small female; bulldog type. April 5, 1916. The aorta was exposed through a median laparotomy wound. An elliptical segment of the aorta was excised, and the resulting defect was closed by a continuous longitudinal suture. Although the lumen of the aorta was diminished about one-third of its diameter, the general health of the animal did not seem to be impaired.

Autopsy.—The dog was killed with chloroform April 12. The specimen showed no evidence of thrombosis.

Gross Examination.—At the point of suture the vessel is surrounded by adventitious tissue about 4 cm. thick. The lumen which is present is but slightly if at all diminished in size. The intima is relatively smooth except just at the suture line where it is finely roughened by what appear to be minute plaques of fibrin.

Microscopic Examination.—The section immediately adjacent to the site of suture shows a considerable infiltration of the adventitious tissue by round cells. The structure of the vessel wall is well preserved. At points there is the suggestion of fibrin formation on the intima. In the lumen of a large sized branch of

the aorta at this point is an extensive thrombus. In the many small vessels in the tissue immediately adjacent, the blood circulation has apparently been free.

Homotransplantation of Carotid Tube to Severed Aorta.

Dog 3, Donor.—April 18, 1916. Both carotids were removed. The carotids were slit longitudinally, and the free lateral borders sutured together so as to form an arterial tube twice the caliber of a single carotid. The dog was killed under ether at the operation.

Dog 4, Host.—Small female fox-terrier. A segment of carotid tube 3 cm. long was transplanted by a biterminal suture between the severed ends of the abdominal aorta. The transplantation was made somewhat difficult by inability to control completely the hemorrhage from the aorta during the suture. The immediate circulation, when the operation was completed, was satisfactory. April 20. Animal died.

Autopsy.—No evidences of leakage. Lumen patent. Sutures plainly visible. The specimen showed evidences of lateral thrombosis.

Gross Examination.—The vessel wall is intact but slightly thinner than the normal vessel adjacent. The longitudinal suture lines which have reconstructed the segments of the carotid into the single tube for transplant are plainly visible, though apparently covered by tissue. The intimal surface of the transplant is but slightly roughened by plaques of fibrin. The transverse suture lines are slightly roughened by minute coagula.

Microscopic Examination.—Section across the transverse suture line shows that the intimal surface is covered here and there by a thin layer of coagulated blood. The transplanted segment is somewhat degenerated and infiltrated by round cells. Section through the center of the transplanted segment shows a thin layer of coagulated blood on the intima, and the vessel wall has partially degenerated though it is physically intact (Fig. 1).

Homotransplantation of a Segment of Aorta to the Aorta.

Dog 5.—Medium sized male mongrel. May 17, 1916. The aorta was exposed by a median laparotomy and liberated for a distance of about 5 cm. Two elastic clamps were then applied and the aorta was severed. A segment about 3 cm. long had been removed about an hour before from a small mongrel dog that had been killed by chloroform. This segment was now interposed between the severed ends of the aorta and united by a biterminal suture. After the circular suture was completed, there was no leakage.

Autopsy.—The dog was killed with chloroform June 5. With the exception of the adhesion of the omentum to one point in the line of suture there were no traces of peritoneal irritation. The peritoneum covering the aorta was absolutely smooth. There was some thickening about the transplanted segment. No thrombosis.

Gross Examination.—The aorta is surrounded by a mass of adventitious tissue measuring about 1 cm. in thickness. At the site of the suture a distinct wall of the vessel is apparent, differentiated from the surrounding tissue. The lumen which is present is somewhat diminished in size at the site of repair. There are no blood coagulations in the lumen. The intima appears relatively smooth.

Microscopic Examination.—There is extensive round cell infiltration of the adventitia in and about the sutures. External to the vessel wall are areas of hemorrhage—the result of operation. The vessel wall at this point is intact and whatever intima is shown in the section is free from blood coagulations.

Transplantation of Fascia to a Lateral Defect in the Aorta.

Dog 6.—Medium sized fox-terrier. May 24, 1916. By a median laparotomy the aorta was exposed and liberated for a distance of about 5 cm. A rectangular clamp was applied, and a section ½ cm. square was removed from the anterior wall. This defect was covered by transplanting a piece of aponeurosis taken from the abdominal wall.

Autopsy.—The dog was killed on June 9 with chloroform. The peritoneal cavity was free from adhesions except for a fibrous deposit about the parietal peritoneum corresponding to the field of operation. There were no evidences of leakage from the aorta, and there was no obstruction or thrombosis. The area of intimal surface corresponding to the transplant was perfectly smooth.

Gross Examination.—The specimen consists of a segment of aorta surrounded at the site of fascial transplant by a mass of adventitious tissue. Cross-section shows that the arterial wall at the transplant is deficient and is replaced by scar tissue. The intimal surface over the site of the transplant is covered with a thin layer of fibrin. This surface is relatively smooth. The vessel lumen is in no way diminished at this point. Adjacent to the patch projecting into the lumen is a rounded mass of coagulum about 1 mm. long, apparently a suture end free in the blood stream covered by this blood clot.

Microscopic Examination.—Between the ends of the sutured artery there is a mass of loosely built connective tissue. The surface is smooth where this granulation tissue is exposed to the blood stream. An intact cellular layer of adapted connective tissue cells forms this smooth surface. There is a small island of fibrin attached at one point. In the depths of the artery wall are sections of several sutures. Around the sutures are cellular aggregations among which are many polymorphonuclear leukocytes. At one point in the tissue, held in the interval between the artery ends, there is a deposit of lime salts suggesting the early stages of ossification. This sutured vessel demonstrates a favorable repair with a satisfactory reestablishment of the blood circulation (Fig. 2).

CONCLUSIONS.

1. Injuries of the abdominal aorta in dogs may be corrected with subsequent perfect restoration of the continuity of the vessel.

2. The complete occlusion of the aorta for a period of 30 minutes is not necessarily followed by serious consequences.

3. In cases in which a portion of the aorta must be resected, an arterial segment taken from another animal can be safely utilized as a transplant.

4. While the reestablishment of the continuity of the severed aorta by the circular suture is possible, the approximation of the severed ends during the suture entails such injury that thrombosis frequently occurs. Therefore, when the aorta is completely severed, the introduction of a transplanted segment is indicated.

5. An arterial tube of increased caliber made of smaller vessels such as the carotid lends itself readily as a transplant to the severed aorta, with a reasonable assurance of reestablishing the continuity of this vessel.

6. Defects in the aorta can be readily corrected by the use of fascial transplants with a minimum danger of thrombosis.

EXPLANATION OF PLATES.

Plate 23.

Fig. 1. Sutured vessel wall. *A*, suture line of vessel wall. Eversion of the coats of the vessel wall in order to bring the intima in contact with the intima at the suture line. There is a parietal coagulation of blood, *B*, into which reparative tissue grows in the final establishment of repair. Compare with Fig. 2, in which growth of tissue is complete, reestablishing the intimal lining of the vessel.

Plate 24.

Fig. 2. Sutured vessel wall. The everted vessel wall, *A*, joins the tissue transplant, *B*. The interval between the vessel wall and tissue transplant is covered by a mass of reparative tissue, *C*, which has grown into a blood coagulum, usually found present in early specimens after vascular suture. Compare with Fig. 1.

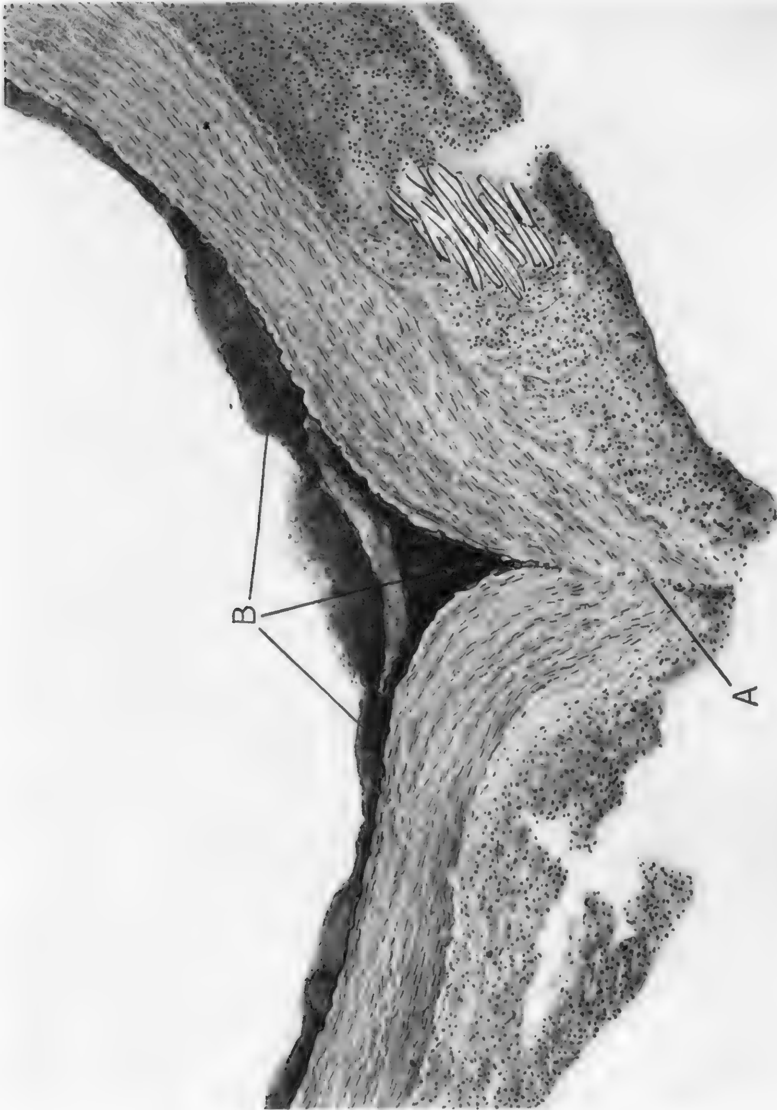


FIG. 1.

(Goodman: Operations on the abdominal aorta.)

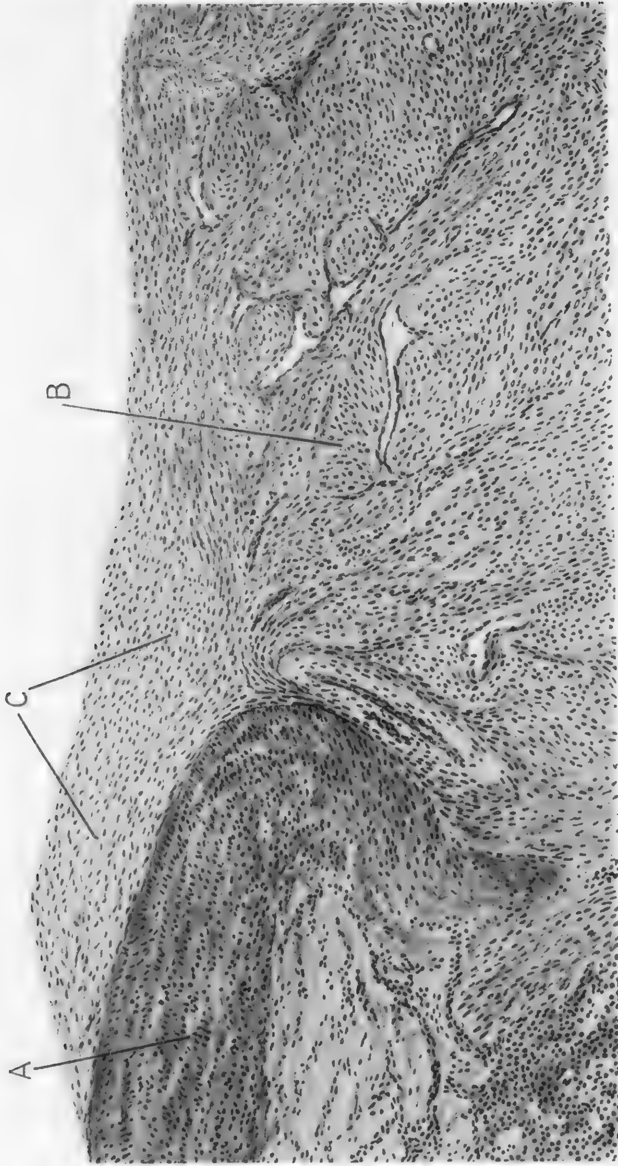


Fig. 2.

(Goodman: Operations on the abdominal aorta.)

MORPHOLOGICAL CHARACTERISTICS AND NOMENCLATURE OF LEPTOSPIRA (SPIROCHÆTA) ICTEROHÆMORRHAGIÆ (INADA AND IDO).

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PLATES 25 TO 29.

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In a previous communication, the writer reported the presence in American wild rats of a spirochete morphologically and immunologically identical with the *Spirochæta icterohæmorrhagiæ* of Inada and his associates and also with the strain isolated by Stokes from cases of infectious jaundice among British soldiers in Flanders.[1] The European strains, which have now been isolated from cases on the British, French, and Italian fronts, as well as from wild rodents captured not only near the battle-lines but in regions remote from them, are undoubtedly strains of the same organism.[2] Jobling and Eggstein[3] have also found the same spirochete recently among wild rats caught in Tennessee.

Just how, in nature, a rat becomes a carrier of the spirochete is not at once apparent. It is not improbable that the contamination of a foodstuff by the urine of an infected rat may transmit the organism to other rats; or the animal may become infected by feeding upon an infected dead rat, since a rat may be experimentally infected by feeding it with an infected foodstuff or with an infected tissue or

[1] Noguchi, H., *J. Exp. Med.*, 1917, xxv, 755.

[2] Costa, S., and Troisier, J., *Presse méd.*, 1916, lxxx, 526, 565. Courmont, J., and Durand, P., *Bull. et mém. Soc. méd. hôp.*, 1917, xli, series 3, 115. Clément, P., and Fiessinger, N., *Presse méd.*, 1916, lxxx, 598. Garnier, M., *Compt. rend. Soc. biol.*, 1916, lxxix, 928. Manine, Cristau, and Plazy, *Compt. rend. Soc. biol.*, 1917, lxxx, 531. Wilmaers, L., and Renaux, E., *Arch. méd. Belges*, 1917, lxx, 115, 207. Dawson, B., and Hume, W. E., *Quart. J. Med.*, 1916–17, x, 90. Zironi, A., and Capone, G., *Sperimentale*, 1917, lxxi, 298. Ascoli, M., and Perrier, S., *Gazz. osp.*, 1916, xxxvii, 1618. Sisto, P., *Sperimentale*, 1917, lxxi, 361. Siccardi, P. D., and Bompiani, G., *Ann. ig.*, 1917, xxvii, 609. Moreschi, C., *Policlinico, Sez. Prat.*, 1917, xxiv, 265. Sampietro, G., *Ann. ig.*, 1917, xxvii, 23.

[3] Jobling, J. W., and Eggstein, A. A., *J. Am. Med. Assn.*, 1917, lxix, 1787.

organ. Whatever the mode of preservation in nature, *Spirochæta icterohæmorrhagiæ* is a common commensal among rodents.

Morphology.

The morphology of this organism has been the subject of much study by its discoverers and by others, but its distinctive feature does not seem to have been recognized. Inada and his associates described the organism as a spirochete with several irregular waves, the entire body being dotted with alternate bright and shadowy portions.[4] Hübener and Reiter, who described a similar picture, apparently believed that the organism had a series of minute knots, and hence gave it the name *Spirochæta nodosa*.[5]

That these investigators overlooked the true structure must have been due either to the difficulty of observing the organism, even under a powerful dark-field illumination, or to the indistinctness of the minute spirals in a stained preparation. It appears as an almost smooth bodied, wavy organism, not unlike *Spironema refringens* when fixed in methyl alcohol and stained with Giemsa's solution (Figs. 1, 2, and 3). As has been said in a previous paper,[1] the natural features of the organism can be well preserved when it is fixed in osmic vapor and then stained over night with Giemsa's solution. In such a preparation it is stained light purple and is seen to consist of a very tightly and regularly wound cylindrical filament tapering to sharply pointed extremities. The filament usually assumes a graceful hook at one or both ends, while the main portion may be straight or slightly bent (Figs. 4 and 5). The number of spirals (not waves) varies considerably according to the length of the specimen, which may be between 3 to 20, 30, or even 40 μ, but the distance between the apices of two spirals measures about 0.5 μ. For example, a specimen measuring 9 μ would have eighteen spirals. The thickness, or diameter of a cross-section, of the organism is nearly uniform until it approaches the terminal portion, which may be so conveniently designated because of its tapering points and its hooked attitude. The number of spirals in the terminal portion appears to

[4] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[5] Hübener and Reiter, *Deutsch. med. Woch.*, 1916, xlii, 1.

be about six in all specimens, and it is this portion which exhibits the greatest tendency to become bent to a semicircle. Unlike various spironemata or treponemata, the spiral amplitude near the extremities is not noticeably less than that of the middle portion of the organism.

In certain specimens the terminal portions are far less intensely stained than the main portion (Figs. 1, 2, and 3). In the majority of specimens, both terminal portions are bent to the same side (Figs. 5, 6, 13, 16, 17, 19, and 22), but in some they form hooks of opposite direction (Fig. 4), unipolar hooks (Figs. 8, 9, 15, and 20), or are not bent at all (Figs. 11 and 12); and some are contorted (Fig. 18). In the less well preserved specimens the spirals are no longer distinct but appear as somewhat more deeply stained dots (Fig. 21). As has already been pointed out, under a powerful dark-field illumination the organism in rapid rotary motion seems to be surrounded by a halo. This may be only an optical effect, but a similar clear zone has been noticed in the stained preparations of some specimens (Figs. 12, 15, 17, and 23).

The dark-field picture of the organism is such that one may mistake the minute spirals for refractive beads arranged diagonally or somewhat obliquely with respect to the axis of the organism (Figs. 24 and 27 to 33), as originally depicted by Inada and his associates and others. But, as has been stated before, with a favorable and powerful illumination, the real structure can be revealed (Figs. 24, 25, and 34).

Only a few of the photomicrographs represent the characteristically hooked forms (Figs. 26 and 28) as actually seen in active rapid rotary motion in a free space, because it was difficult to photograph the organisms in motion, and as soon as motion ceases many of them lose the typical hooks. The large wavy undulations, however, (not the elementary spirals), as assumed by the organisms when penetrating semifluid medium, are well shown in some of the specimens at rest (Figs. 27 and 29 to 33). The remarkable flexibility of the organism in a semisolid medium is also shown (Figs. 27, 32, and 33). These minute filamental organisms dart through the soft medium with great rapidity, first in one direction and then in another, searching for a loose spot which they can pierce through. When encountering

an impenetrable obstacle they reverse their progression and start anew. A striking sight is thus presented by these little vermicular organisms darting in all directions. A vibratory motion of the free portion of the organism results when it is extricating itself from an entanglement. In an emulsion of infected liver one may encounter a tangle of several actively motile organisms (Figs. 24 and 25), while in a culture several weeks old a mass of hundreds of motile spirochetes may be found (Fig. 35).

The European (Figs. 36 to 57) and Japanese (Figs. 58 to 68) strains have all the morphological features given for the American strain. It might be mentioned here that the elementary spirals in the terminal portion are much smaller in number and less regular in the stained specimens of the European strain, but this may be due to imperfect fixation of the organism, because under the dark-field microscope the spirals are equally close and regular.

Classification.

Characteristics of Different Genera of Spiral Organisms.

In order to determine the systematic position of the organism of infectious jaundice, it may be well to review here the characteristics of various genera of spiral organisms. Through the recent investigations of Gross,[6] Zuelzer,[7] Dobell,[8] Gonder,[9] Swellengrebel,[10] and others, the organism for which Ehrenberg created the term *Spirochæta* in 1838 is now known to be distinct from the majority of so called spirochetes. It consists of a long, highly flexible, central axial filament surrounded by a regularly wound layer of protoplasm, usually of great length (200 to 500 μ), and is free living in fresh or marine water (Fig. 108). Neither a membrane nor a flagellum is present. Multiplication takes place by transverse fission. The organism

[6] Gross, J., *Centr. Bakteriol., 1te Abt., Orig.*, 1912, lxv, 83.

[7] Zuelzer, M., *Arch. Protistenk.*, 1912, xxiv, 1.

[8] Dobell, C., *Proc. Roy. Soc. London, Series B*, 1912, lxxxv, 186.

[9] Gonder, R., Spironemacea (Spirochaeten), in von Prowazek, S., Handbuch der pathogenen Protozoen, Leipsic, Liefg. 6, 1914, 671.

[10] Swellengrebel, N. H., *Ann. Inst. Pasteur*, 1907, xxi, 448; *Compt. rend. Soc. biol.*, 1907, lxii, 213.

creeps along the surface of an object but does not swim. Only four species belonging to this genus have been described. The organism under discussion does not belong to it.

Cristispira and Saprospira.—For a limited variety of coarse, actively motile spiral organisms infesting the crystalline styles of certain mollusca, the genus *Cristispira* was proposed by Gross in 1910.[11] The characteristic features are: the presence of a membranous structure running spirally from one end of the body to the other, assuming the aspect of a crista or ridge; the chambered structure of the body; the absence of a terminal filament; and the existence of a strong, flexible membrane (Figs. 104 to 106). According to Gross, reproduction may be effected by multiple transverse fission or sporulation, though I have failed to confirm the occurrence of sporulation. More than a dozen species have been described, but from personal observations I doubt whether these so called species are sufficiently characteristic to be so distinguished. The type organism was first described by Certes in 1882[12] as found in oysters, and was known as *Spirochæta* or *Trypanosoma balbianii*. Another genus, *Saprospira*, was proposed by Gross in 1912[13] for a few varieties of spiral organisms in mussels which differed from the cristispiræ in not having a crista (Fig. 107). The organism in question, however, belongs to neither of these genera.

Spironema and Treponema.—Next in order is the large group of small parasitic spiral organisms commonly called spirochetes. Among them are the causative agents of syphilis and yaws (Figs. 69 to 72 and 103) and of relapsing fevers in man and animals (Figs. 94 to 100), non-pathogenic parasites in certain rodents, and various saprophytic types on or about the oral, alimentary, or genital mucous membranes (Figs. 73 to 93). Their essential feature is a spiral flexible body with terminal filaments, but no undulating membrane. They seem to multiply by transverse as well as longitudinal fission. The rigidity of the curves differs greatly in different organisms, some becoming almost flat at death or constantly changing the waves by oscillatory undulation, others retaining their regular curves even during motion or after death. The whole group has been called *Spirochætæ* or *Spirilla*, in spite of the

[11] Gross, J., *Mitt. zool. Station Neapel*, 1910–13, xx, 41.

[12] Certes, A., *Bull. Soc. zool. franc.*, 1882, vii, 347; 1891, xvi, 95.

[13] Gross, J., *Mitt. zool. Station Neapel*, 1910–13, xx, 188.

fact that they have no affinity with the real spirochete or non-flexible spirillum. Gross includes them in the genus *Spironema*, a term introduced by Vuillemin[14] in 1905 to distinguish Schaudinn's organism of syphilis from those with less rigid spirals. Dobell,[8] however, believes that the term *Treponema*, as proposed by Schaudinn[15] himself in 1905 for his organism, should be employed to designate all these minute parasitic varieties. Gonder[9] takes a more conservative stand and upholds the distinction made by Schaudinn between the treponema type and that with less constant curves. For example, Gonder retains the genus *Spironema* for the latter and *Treponema* for the former type. I agree with Gonder in this respect, as the general features are sufficiently distinct to enable one to differentiate the two groups.

Nomenclature of Leptospira (Spirochæta) icterohæmorrhagiæ.

The striking differences between the organism of infectious jaundice and all the other so called spirochetes, or rather spironemata and treponemata, are apparent at a glance. The closely set, regular spirals of the organism of Inada and Ido remain unmodified during its rotary, spinning motions in a free space and when it is piercing a semisolid medium. While in motion in a free space, the whole body appears tightly drawn into a straight line, except for the usual hook formation of one or both terminal portions. When one end is extended and straight and the other semicircularly hooked, the organism usually progresses in the direction of the straight portion and seems to be propelled from the rear by the rotating hook (Figs. 8, 9, 15, and 20). A specimen with both ends hooked remains stationary in spite of its rapid rotary motions (Figs. 13, 16, and 19). By straightening one end or the other alternately, the organism changes its progression from one direction to the opposite one. When the organism penetrates a soft medium, changing direction very rapidly, it seldom shows hooked ends (Figs. 11, 12, 29, 30, 32, and 33). In this sort of movement the body assumes wide wavy undulations such as are seen in an active specimen of *Spironema refringens*. The behavior of the organism in semisolid medium is different from that

[14] Vuillemin, P., *Compt. rend. Acad.*, 1905, cxl, 1567.
[15] Schaudinn, F., *Deutsch. med. Woch.*, 1905, xliii, 1728.

in a free space. The persistence of the minute elementary spirals at all times is a feature which distinguishes this organism from any treponema or spironema. The depth of the spirals does not exceed the diameter of the body, a fact unknown among other so called spirochetes. A diligent search by means of various staining methods, as well as by dark-field illumination applied to cultures of different ages, has not demonstrated a terminal flagellum or peritrichal flagella or membranes. It is well to recall at this point that in old cultures of all the spironemata or treponemata I have isolated I have been able to demonstrate the presence of a terminal filament, even when it was observed with difficulty in uncultivated specimens. This organism, however, as far as we know at present, moves by means of its terminal portions. Moreover, unlike spironemata and treponemata, it withstands the action of 10 per cent saponin. Clearly it does not belong to either of these genera, but must remain in a class by itself until other similar organisms come to our observation. The nearest approach to it in morphological and biological respects is *Spirochæta biflexa*, which was isolated by Wolbach and Binger[16] in 1914 from a filtrate of stagnant water taken from the shore of a fresh water pond near Boston. There is a great similarity between the two organisms. Both are filterable through Berkefeld filters. Wolbach and Binger did not succeed in obtaining a second generation in culture, and no tests of pathogenicity for experimental animals were made.

For the reasons which have been discussed, it seems justifiable to include the type of organism in question under *Leptospira* (λεπτός fine, + σπεῖρα, coil), as has already been proposed.[1]

The genera with their type organisms are presented below. The measurements of each of these representative members and the characteristic features used for identification of the genera are considered. There is little difficulty in distinguishing *Spirochæta*, *Saprospira*, *Cristispira*, and *Leptospira* from one another. But the distinction between *Spironema* and *Treponema* depends chiefly upon the rigidity and regularity of the spirals which are characteristic of the treponemata. Under natural conditions this difference is so marked that there should be no confusion in classification, but under cultural

[16] Wolbach, S. B., and Binger, C. A. L., *J. Med. Research*, 1914, xxx, 23.

conditions the spirals of the spironemata acquire such rigidity and regularity that they, too, may be called treponemata. Dobell[8] and Gross,[6] independently of each other, and without any knowledge as to the morphological modifications due to cultivation, regarded the distinction between *Treponema* and *Spironema* as insufficient to maintain two separate genera, and Dobell chose the term *Treponema* and Gross *Spironema* for the same group of organisms. In my opinion the characteristics of *Treponema* and *Spironema*, under natural conditions, are sufficiently pronounced to justify retaining the two terms in classification. Neither *Treponema* nor *Spironema* has any feature which is likely to be confused with those of the other four genera referred to above. Text-fig. 1 shows the types mentioned below.

Genus.—Spirochæta (Ehrenberg, 1838). *Type Organism.—Spirochæta plicatilis* (Ehrenberg, 1838) (Fig. 108). *Measurements.*—Length, 100 to 500 μ; blunt end. Diameter, 0.5 to 0.75μ; cylindrical. Spiral amplitude, 2 μ; regular. Spiral depth, 1.5 μ; regular. Waves, several, large, inconstant, irregular. *Axial Filament.*—Distinct in stained specimens; flexible; elastic. *Chambered Structure.*—Absent. *Membrane.*—Absent. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Free living in fresh or marine water. *Other Species.—Plicatilis marina, plicatilis eustrepta, stenostrepta, daxensis. Staining Properties of Axial Filament and Cell Membrane.*—Axial filament consists of chitin or cutin-like substance. Stains violet by Giemsa's solution and gray by iron-hematoxylin. *Staining Properties of Body.*—Plasmic spirals of the body stain with eosin, rubin, etc. Contain volutin granules. *Trypsin Digestion.*—Axial filament resistant. *Bile Salts* (*10 Per Cent*).—Becomes shadowy pale but is not dissolved. *Saponin* (*10 Per Cent*).—Lives 30 minutes. Later becomes shadowy, but is not dissolved.

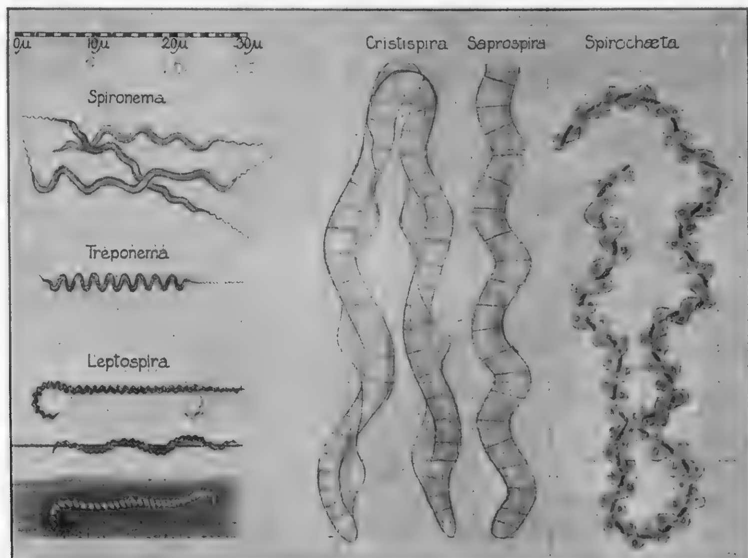
Genus.—Saprospira (Gross, 1911). *Type Organism.—Saprospira grandis* (Gross, 1911). *Measurements.*—Length, 100 to 120 μ; obtuse end. Diameter, ?μ; cylindrical. Waves, large, inconstant, shallow, irregular, 3 to 5 in number. Sometimes almost straight. *Axial Filament.*—Absent. *Chambered Structure.*—Present. *Membrane.*—Distinct, flexible, elastic. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Free living in foraminiferous sand. *Other Species.—Nana.*

Genus.—Cristispira (Gross, 1910). *Type Organism.—Cristispira balbianii* (Certes, 1882) (Figs. 104 and 105). *Measurements.*—Length, 45 to 90 μ; obtuse end. In stained preparations the end may be sharply pointed, but this is due

CORRECTION.

On page 583, Vol. xxvii, No. 5, May 1, 1918, the scale of Text-fig. 1 should read 0, 5, 10, and 15 μ instead of 0, 10, 20, and 30 μ.

to shrinkage by fixing reagents. Diameter, 1 to 1.5 μ; cylindrical. Waves, 2 to 5, sometimes more, large, irregular, shallow. In a dying specimen the waves may be more numerous and regular. *Axial Filament.*—Absent. *Chambered Structure.*—Present. *Membrane.*—Distinct, flexible, elastic. *Crista.*—Present, a ridge-like membrane. Spirally wound body. *Terminal Finely Spiral Filament.*—Absent. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse. *Habitat of Genus.*—Parasitic in the alimentary canals of shell-fish. *Other Species.*—*Ostreæ, anodontæ, modiolæ, veneris, tapetos, chamæ,* etc. *Staining Prop-*



TEXT-FIG. 1. Diagram contrasting the characteristic features and relative proportions of *Spironema, Treponema, Cristispira, Saprospira, Spirochæta,* and *Leptospira.* The scale in microns is given in the upper left-hand corner of the figure.

erties of Axial Filament and Cell Membrane.—Membrane behaves like chitin or cutin substance. Stains violet by Giemsa's solution and light gray by iron-hematoxylin. *Staining Properties of Body.*—The body is alternately stained red and bluish violet and the crista red by Giemsa's solution. Iron-hematoxylin brings out sharp septa and a layer of chromatin granules. *Trypsin Digestion.*—Membrane resistant. Crista and chambers disappear. *Bile Salts* (*10 Per Cent*).—Crista quickly destroyed. Body not attacked. *Saponin* (*10 Per Cent*).—Crista becomes fibrillar, then indistinct. Body not affected.

Genus.—Spironema (Vuillemin, 1905). *Type Organism.—Spironema recurrentis* (Lebert, 1874[17]) (Figs. 94 to 96). *Measurements.*—Length, 8 to 16 μ; pointed ends. Diameter, 0.35 to 0.5 μ; cylindrical or slightly flattened. Spirals, large, wavy, inconstant, about five in number. Closer and more regular in cultures. *Axial Filament.*—Probably present. *Chambered Structure.*—Absent. *Membrane.*—Delicate, flexible, double contoured. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Present, easily seen in cultures. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse, possibly also longitudinal. *Habitat of Genus.*—Numerous pathogenic and non-pathogenic varieties. *Other Species.—Carteri, kochi, novyi, duttoni, berbera, aegyptica, gallinarum, anserina, theileri, equi, muris, eugyratum, microgyratum, buccalis, refringens,* etc. *Staining Properties of Axial Filament and Cell Membrane.*—Membrane difficult to differentiate. *Staining Properties of Body.*—Stains violet by Giemsa's solution. *Bile Salts* (*10 Per Cent*).—Disintegration complete. *Saponin* (*10 Per Cent*).—Immobilized in 30 minutes then broken up in a few hours. In some there is an axial filament laid bare.

Genus.—Treponema (Schaudinn, 1905). *Type Organism.—Treponema pallidum* (Schaudinn and Hoffmann, 1905[18]) (Figs. 69 to 72 and 103). *Measurements.*—Length, 6 to 14 μ; pointed ends. Diameter, 0.25 to 0.3 μ; cylindrical. Spiral amplitude, 1 μ; regular, rigid. Spiral depth, 0.8 to 1 μ; very constant. Waves, one or more slight undulating curves may be present. *Axial Filament.*—Doubtful. The whole seems to consist of a spirally wound axial filament. *Chambered Structure.*—Absent. *Membrane.*—Doubtful; if there is one it must be flexible. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Present. Easily seen in cultures. *Flagella.*—Absent. *Highly Motile End Portion.*—Absent. *Division.*—Transverse or possibly also longitudinal. *Habitat of Genus.*—Two pathogenic and several harmless parasites. *Other Species.—Pertenue, microdentium, macrodentium, mucosum, calligyrum, minutum. Staining Properties of Axial Filament and Cell Membrane.*—Membrane not recognizable. *Staining Properties of Body.*—Stains pink by Giemsa's solution. *Trypsin Digestion.*—Resists digestion for many days. *Bile Salts* (*10 Per Cent*).—Disintegration complete. *Saponin* (*10 Per Cent*).—Broken up in time.

Genus.—Leptospira (Noguchi, 1917). *Type Organism.—Leptospira icterohæmorrhagiæ* (Inada and Ido, 1914) (Figs. 1 to 68, 101, and 102). *Measurements.*—Length, 7 to 9 to 14 μ; exceptionally 30 to 40 μ; pointed ends. Diameter, 0.25 to 0.3 μ; cylindrical. Spiral amplitude, 0.45 to 0.5 μ; regular, rigid. Spiral depth, 0.3 μ; regular. Waves, one or more gentle wavy curves throughout the entire length. When in a free space, one or both ends may be semicircularly hooked, while in semisolid media the organism appears serpentine, waved, or bent. Its flexibility is most striking. *Axial Filament.*—Not recognized. *Chambered Struc-*

[17] Lebert, H., Rückfallstyphus, Flecktyphus und Cholera, in von Ziemssen, H., Handbuch der speciellen Pathologie und Therapie, Leipsic, 1874, ii, 267.

[18] Schaudinn, F., and Hoffmann, E., *Arb. k. Gsndhtsamte.*, 1905, xxii, 527.

ture.—Absent. *Membrane.*—Not recognized. *Crista.*—Absent. *Terminal Finely Spiral Filament.*—Not recognized. *Flagella.*—Absent. *Highly Motile End Portion.*—Well developed in the last six to eight spirals. *Division.*—Transverse. *Habitat of Genus.*—One pathogenic and one possibly non-pathogenic variety known. *Other Species.*—*Biflexa* (Wolbach and Binger). *Staining Properties of Axial Filament and Cell Membrane.*—Membrane not recognizable. *Staining Properties of Body.*—Stains reddish violet by Giemsa's solution. *Bile Salts* (*10 Per Cent*).—Easily dissolved. *Saponin* (*10 Per Cent*).—Completely resistant.

The comparative dimensions of these representative organisms may be shown by putting side by side the diameter, spiral amplitude, spiral depth, and length of each, taking the diameter of the finest member, *Leptospira icterohæmorrhagiæ*, as a unit of comparison (Table I).

TABLE I.

Organism.	Thickness.	Spiral amplitude.	Spiral depth.	Length.
Leptospira icterohæmorrhagiæ...	1 (0.25 μ)	2 (0.5 μ)	1.2 (0.3 μ)	56 (14 μ)
Treponema pallidum..........	1.2 (0.3 μ)	4 (1 μ)	3.6 (0.9 μ)	48 (12 μ)
Spironema obermeieri..........	2 (0.5 μ)	12 (3 μ)	6 (1.5 μ)	32 (8 μ)
Cristispira balbianii...........	5 (1.2 μ)	60 (15 μ)	24 (6 μ)	200 (50 μ)
Saprospira grandis............	5 (1.2 μ)	32 (8 μ)	8 (2 μ)	400 (100 μ)
Spirochæta plicatilis...........	3 (0.75 μ)	18 (4.5 μ)	6 (1.5 μ)	600 (150 μ)

One may obtain the comparative proportions for each genus by using the diameter of its representative member as a unit of comparison, as in Table II.

TABLE II.

Organism.	Thickness.	Spiral amplitude.	Spiral depth.	Length.
Spirochæta plicatilis...........	1 (0.75 μ)	6 (4.5 μ)	2 (1.5 μ)	200 (150 μ)
Saprospira grandis............	1 (1.2 μ)	7 (8 μ)	1.8 (2 μ)	83 (100 μ)
Cristispira balbianii...........	1 (1.2 μ)	13 (15 μ)	5 (6 μ)	41 (50 μ)
Spironema obermeieri..........	1 (0.5 μ)	6 (3 μ)	3 (1.5 μ)	16 (8 μ)
Treponema pallidum..........	1 (0.3 μ)	3.3 (1 μ)	3 (0.9 μ)	40 (12 μ)
Leptospira icterohæmorrhagiæ...	1 (0.25 μ)	2 (0.5 μ)	1 (0.25 μ)	56 (14 μ)

The proportions are distinctive for each genus, and form, with other differentiating features already discussed, a fairly well established basis for the classification of these spiral organisms, hitherto so indis-

criminately called by the general name of spirochetes. It would be desirable, in describing a new spiral organism, to place it in one of the six classes discussed, since under the vague name of spirochete no one can visualize the actual features of the organism in question, while if it is called *Leptospira,* for example, certain definite features are connoted, and confusion with other so called spirochetes is avoided. This is particularly important when one is examining specimens of urine such as those from certain cases in which a *Leptospira* or a *Treponema* may be present alone or together, as in a study of trench infections. Patterson[19] and Nankivell and Sundell[20] discovered the latter type in cases of trench fever of unknown origin, while the former has been found responsible for a number of cases of various trench affections.[21,22]

A brief note may be made of the relation of *Leptospira* to a comparatively minute species of spirochete, *Spirochæta stenostrepta*, described by Zuelzer[7] (Figs. 109 and 110). The organism was found in stagnant water with *Spirochæta plicatilis*. It has a diameter of 0.25 μ and a length of 20 to 60 μ, seldom reaching a length of 200 μ. In a short specimen which measured 13 μ there were eleven spirals. In life an axial filament was recognized. Here the leptospira can be distinguished by its lack of an axial filament and its closer spirals. In the latter respect certain oscillatorial organisms such as *Spirulina vesicolor* (Figs. 111 and 112), or *Spirulina tenuissima* have a superficial resemblance to leptospira, but their multicellular structure, which can be demonstrated by subjecting them to a preliminary treatment with trypsin solution before staining, shows them to be very different. Each coil here represents an individual cell separated from the adjoining cells by walls. The spirulina has blunt ends and does not exhibit the active, brusque movements characteristic of leptospira.

[19] Patterson, S. W., *J. Roy. Army Med. Corps*, 1917, xxix, 503.
[20] Nankivell, A. T., and Sundell, C. E., *Lancet*, 1917, ii, 672, 836.
[21] Couvy, L., and Dujarric, R., *Compt. rend. Soc. biol.*, 1918, lxxxi, 22.
[22] Dudgeon, L. S., *Lancet*, 1917, ii, 823.

SUMMARY.

The present study deals with the morphology and systematic position of the causative agent of infectious jaundice. There are several features which are not found in any of the hitherto known genera of Spirochætoidea which led me to give this organism an independent generic name, *Leptospira*, denoting the peculiar minute elementary spirals running throughout the body. The absence of a definite terminal flagellum or any flagella, and the remarkable flexibility of the terminal or caudal portion of the organism are other distinguishing features. Unlike all other so called spirochetes the present organism resists the destructive action of 10 per cent saponin.

A detailed comparative study of related genera, including *Spirochæta*, *Saprospira*, *Cristispira*, *Spironema*, and *Treponema*, has been given with the view of bringing out more strongly the contrast between them and the new genus.

A study has been made to discover whether any differential features exist among the strains of *Leptospira icterohæmorrhagiæ* derived from the American, Japanese, and European sources, but none has been found.

It is hoped that the creation of a new genus may facilitate a more exact morphological description than has hitherto been possible, due to the vague use of the term *Spirochæta* which indiscriminately covered at least six large genera of spiral organisms.

EXPLANATION OF PLATES.

Plate 25.

Figs. 1 to 23 show the morphological features of the American strain of *Leptospira icterohæmorrhagiæ* in stained preparations.

Fig. 1. *Leptospira icterohæmorrhagiæ* in the blood of an experimentally infected guinea pig, showing irregular refringent waves, but no minute elementary spirals. Methyl alcohol fixation and Giemsa's solution. × 1,000.

Fig. 2. The same in a liver emulsion from a similar animal. Except for the few moderate undulations of the body, there is no indication here that these are spiral organisms. Methyl alcohol fixation and Giemsa's solution. × 1,000.

Fig. 3. The same in a kidney emulsion. Fixation and staining the same as above. × 1,000.

FIG. 4. The same in a blood specimen of an infected guinea pig. Fixation and staining the same as above. × 1,000.

Figs. 1 to 4 are intended to show the appearance of the leptospiræ in an air-dried specimen, fixed with methyl alcohol, and stained with Giemsa's solution. They do not show any elementary spirals and appear as smooth, somewhat wavy filaments.

FIGS. 5 to 11. *Leptospira icterohæmorrhagiæ* in stained preparations from a culture in its first generation on the 5th day. They were fixed when moist by osmic acid vapor for 2 minutes, then hardened in absolute alcohol for 30 minutes, and after being thoroughly washed in distilled water, were stained over night with Giemsa's solution (1:20 dilution). In these preparations there were many instances where the fixation and staining were not so satisfactory as in the specimens shown in these photomicrographs. A careful examination makes possible recognition of the closely set, minute, regular spirals throughout the entire length of the organism. With a magnification of 1,000 they are almost too minute to enable one to count the number of the spirals. × 1,000.

FIGS. 12 to 23. *Leptospira icterohæmorrhagiæ* magnified 3,000 times, which brings out the features more distinctly. All except Figs. 21 to 23 show the elementary spirals well. There are ten to twelve spirals to every 5 μ, making the distance between the apex of one spiral to that of the next about 0.5 μ. The terminal portions of the organisms are recognized by the gradually decreasing diameter and the coloration, which is lighter than that of the main portion of the body. These end portions seem to possess about six elementary spirals and measure about 3μ in length. They exhibit remarkable activity and flexibility and serve as propellers in progression in free space and as feelers in guiding the organism through a semisolid medium. Note Fig. 12.

Fig. 18 shows a specimen fixed probably during a somersault movement. The elementary spirals appear as dimly stained cross bars (imperfect fixation).

Fig. 21 shows three organisms attaching themselves to a red corpuscle. The spirals are not distinctly brought out, but one recognizes them as more intensely stained dots, arranged obliquely with respect to the optical axis of the organism.

Fig. 22 (also Figs. 13 and 19) shows a specimen fixed while rotating on its axis in a free space. The organism was otherwise stationary, as shown by its symmetrically bent hooks. Compare with Fig. 20, which has one hook, and therefore must be proceeding in the direction of the straight end.

The two specimens in Fig. 23 show no definite direction of progression. The spirals, though not well fixed, are fixed sufficiently for recognition.

In Figs. 12, 15, 17, 21, and 23, there is a clear space, or halo (about 0.15 μ wide) about the organisms along the entire length. Whether this clear zone, or halo, indicates the presence of a less chromatic membrane enveloping the organisms or is merely due to the dispersion of particles (culture media) from their immediate neighborhood by their rotary movements cannot yet be determined.

PLATE 26.

FIGS. 24 to 35. Specimens of the American strain of *Leptospira icterohæmorrhagiæ* as seen under the dark-field microscope.

Fig. 24. The organisms in a liver emulsion of an experimentally infected guinea pig. They are in resting position and show no characteristic hooked ends. One isolated leptospira has both ends hooked, but not typically, as it would be while actively rotating or progressing in a free space. The spirals appear as regularly set cross bands. × 1,000.

Figs. 25 and 26. A higher magnification of the same specimens. The finely set regular spirals are distinctly shown at the right in Fig. 24, and the cross-barred or dotted aspect of the spirals is shown in the other two of the same figure and also in Fig. 26. × 3,000.

Fig. 27 (× 1,000) and Figs. 28, 29, and 30 (× 3,000) show the leptospiræ in the kidney emulsion of an infected guinea pig. Except for the specimen at the center of Fig. 27, the organisms are in undulatory positions, with gracefully wound, rather loose waves. This position almost always indicates that the organisms are in a semisolid medium, which they are penetrating by means of spiral propulsion. They often remain in the same position for some time before renewing their efforts to extricate themselves. Their dotted or cross-barred appearance remains unmodified under these circumstances.

Fig. 31 (× 1,000) and Fig. 32 (× 3,000) show similar but more pronounced characteristic features.

Fig. 34. The minute elementary spirals are plainly seen in the three entangled leptospiræ in the right upper corner, while in three organisms of Fig. 33 they are recognizable only as dots or bars. × 3,000.

Fig. 35. A large mass of leptospiræ in a fluid culture 3 weeks old. They grow considerably longer in such a medium and form a mass of entangled organisms having the same minute elementary spirals as uncultivated specimens. × 1,000.

PLATE 27.

Figs. 36 to 57 represent the British strain (Stokes) of *Leptospira icterohæmorrhagiæ*.

FIG. 36. A Fontana preparation of the leptospiræ in the liver emulsion of an infected guinea pig. The elementary spirals can hardly be distinguished. × 1,000.

FIG. 37. A badly fixed osmic acid-Giemsa preparation, in which one of the organisms on the extreme right appears as a negative image with minute elementary spirals well brought out. The dye settled about the leptospira without staining the organism itself. × 1,000.

FIGS. 38 to 43. A preparation better fixed with osmic acid vapor and well stained with Giemsa's solution. The leptospiræ were cultivated 7 days at 28°C. In none of them is there any difficulty in discerning the individual elementary

spirals throughout the entire length of the organism. Perhaps owing to imperfect fixation, the elementary spirals in the terminal portions are less numerous and the spiral depth is shallower than in the main portion, which also takes on a more intense stain. In the majority of specimens the spiral amplitude of the main portion is about the same as that of the American or the Japanese strain (0.5 μ). There are a few specimens, however, which measure 0.6 μ from one spiral to the next. × 1,000.

FIGS. 44 to 52. The same. × 3,000.

FIGS. 53 to 57. Dark-field views of the leptospira. Figs. 53, 56, and 57 are from a fluid medium, and Figs. 54 and 55 from a semisolid medium. × 1,000.

PLATE 28.

FIGS. 58 to 68. Dark-field views of the Japanese strain of *Leptospira icterohæmorrhagiæ* from a 7 day culture on semisolid medium. Figs. 58 to 62 are magnified 1,000 times and Figs. 63 to 68, 3,000 times. These photographs show the remarkable flexibility of the tight, elementary spirals of the organisms. The numerous circularly coiled specimens suggest the peculiar hoop-like coiling form of some specimens of *Cristispira balbianii* in the crystalline styles of oysters.

FIGS. 69 to 72. Dark-field views of *Treponema pallidum* which are given here for comparison with the leptospiræ. Their larger spiral amplitude and spiral depth, and their rigidity are sufficiently differentiating. Figs. 69 and 71 are magnified 1,000 times, and Figs. 70 and 72, 3,000 times.

PLATE 29.

Some of these photomicrographs are from stained and some from dark-field preparations. They are reproduced here to illustrate the differential characteristics of several constituent genera of the family of Spirochætoidea (Dobell). × 1,000.

Treponema Group.

FIGS. 73 to 76. Dark-field views of a minute treponema (*Treponema minutum, n. sp.*) found in a smegma. Their average spiral amplitude is 0.9 to 1 μ, spiral depth, 0.2 to 0.5 μ, average number of spirals, eight to ten in 7 to 9 μ, and thickness, 0.3 μ.

FIGS. 80 to 83. *Treponema calligyrum* in smegma. Spiral amplitude, 1.75 μ, depth, 0.5 to 1 μ, four to seven spirals in 7 to 12 μ, thickness, 0.4 to 0.5 μ.

FIG. 84. The same from a culture.

FIG. 85. *Treponema microdentium* from the mouth.

FIG. 86. The same from a culture.

FIG. 88. *Treponema macrodentium* from a culture.

FIGS. 92 and 93. A treponema from the urine of a child, resembling the smallest smegma treponema (Figs. 73 to 76).

Spironema Group.

FIGS. 77 and 78. *Spironema refringens* from smegma. Spiral amplitude, 2 to 2.75 μ; spiral depth, 0.5 to 1.5 μ; four to eight spirals in 11 to 16 μ; thickness, 0.7 μ.

FIG. 79. The same from a culture.

FIG. 87. *Spironema vincenti* from the mouth.

FIGS. 89 to 91. *Spironema buccalis* from the mouth. Spiral amplitude, 2.75 to 3.7 μ; spiral depth, 0.7 to 1 μ; four to seven and one-half spirals in 11 to 17 μ; thickness, 0.5 to 1 μ.

FIGS. 94 and 96. *Spironema recurrentis* in a culture.

FIG. 95. The same in the blood of an infected mouse.

FIG. 97. *Spironema duttoni* in a culture.

FIG. 98. *Spironema kochi* in a culture.

FIG. 99. *Spironema gallinarum* in a culture.

FIG. 100. *Spironema novyi* in the blood of an infected rat.

Leptospira Group.

FIG. 101. *Leptospira icterohæmorrhagiæ*, American strain. × 1,000.

FIG. 102. *Leptospira icterohæmorrhagiæ*, Japanese strain. × 1,000.

FIG. 103. *Treponema pallidum* for comparison. Same magnification.

Cristispira Group.

FIG. 104. Dark-field view of *Cristispira balbianii* from oysters obtained near Woods Hole.

FIG. 105. The same. Osmic acid fixation. Stained with Giemsa's solution.

FIG. 106. *Cristispira veneris* (?) from clams obtained near Long Island Sound. Sublimate alcohol fixation and Heidenhain's iron-hematoxylin.

Saprospira Group.

FIG. 107. An organism possibly belonging to this genus. It was cultivated by me from oysters obtained near New York.

Spirochæta Group.

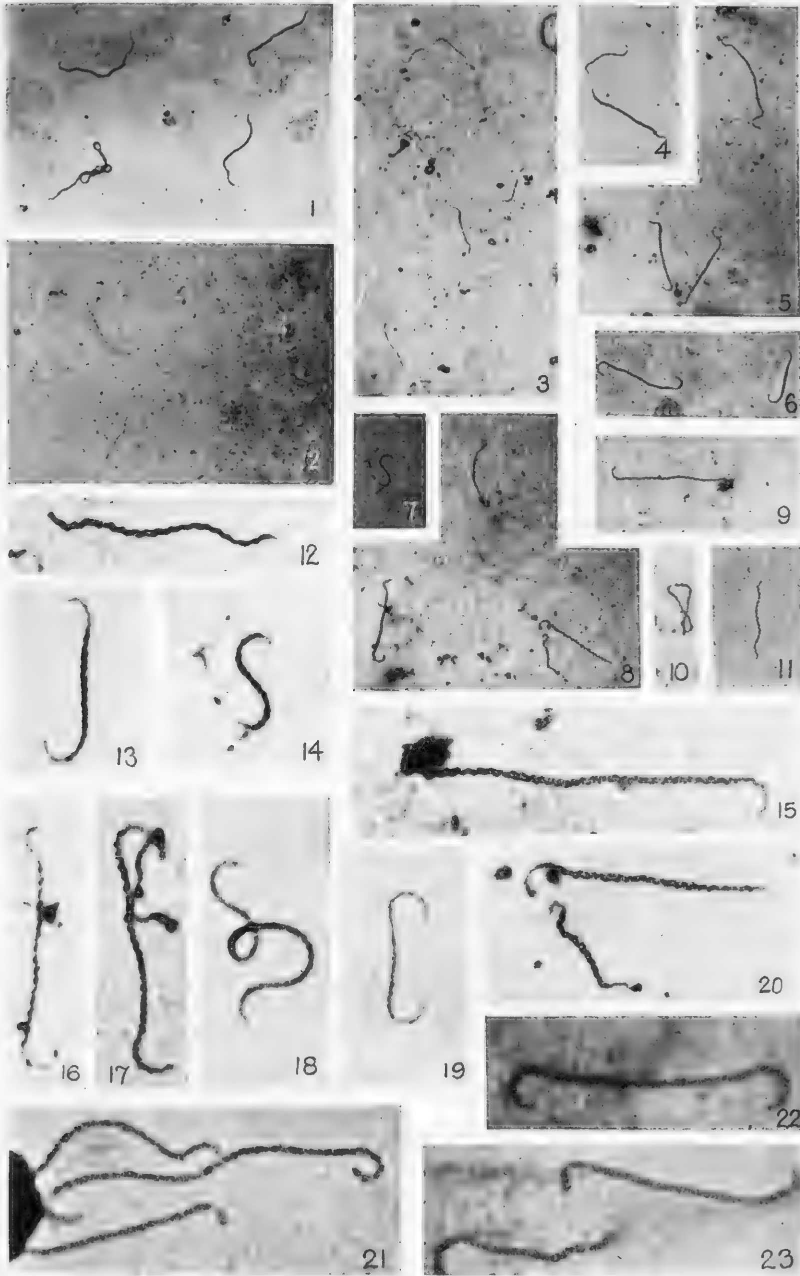
FIG. 108. *Spirochæta plicatilis.* Sublimate acetic-acid-alcohol fixation and iron-hematoxylin (after Zuelzer).

FIGS. 109 and 110. *Spirochæta stenostrepta* (after Zuelzer).

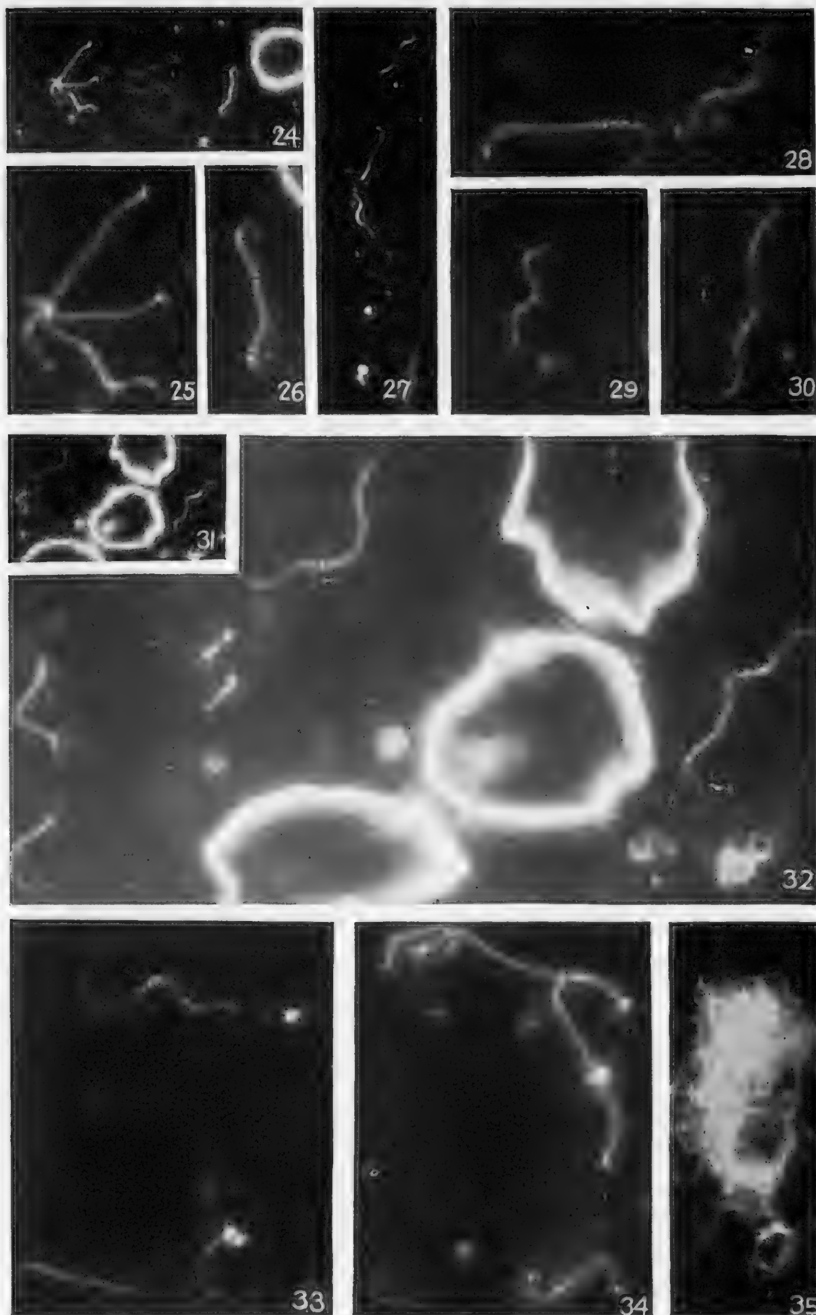
Spirulina Group.

Fig. 111. *Spirulina vesicolor.* This organism does not belong to the family of Spirochætoidea, but on account of its close spirals it is shown here. Iodine-alcohol and Delafield hematoxylin (after Zuelzer).

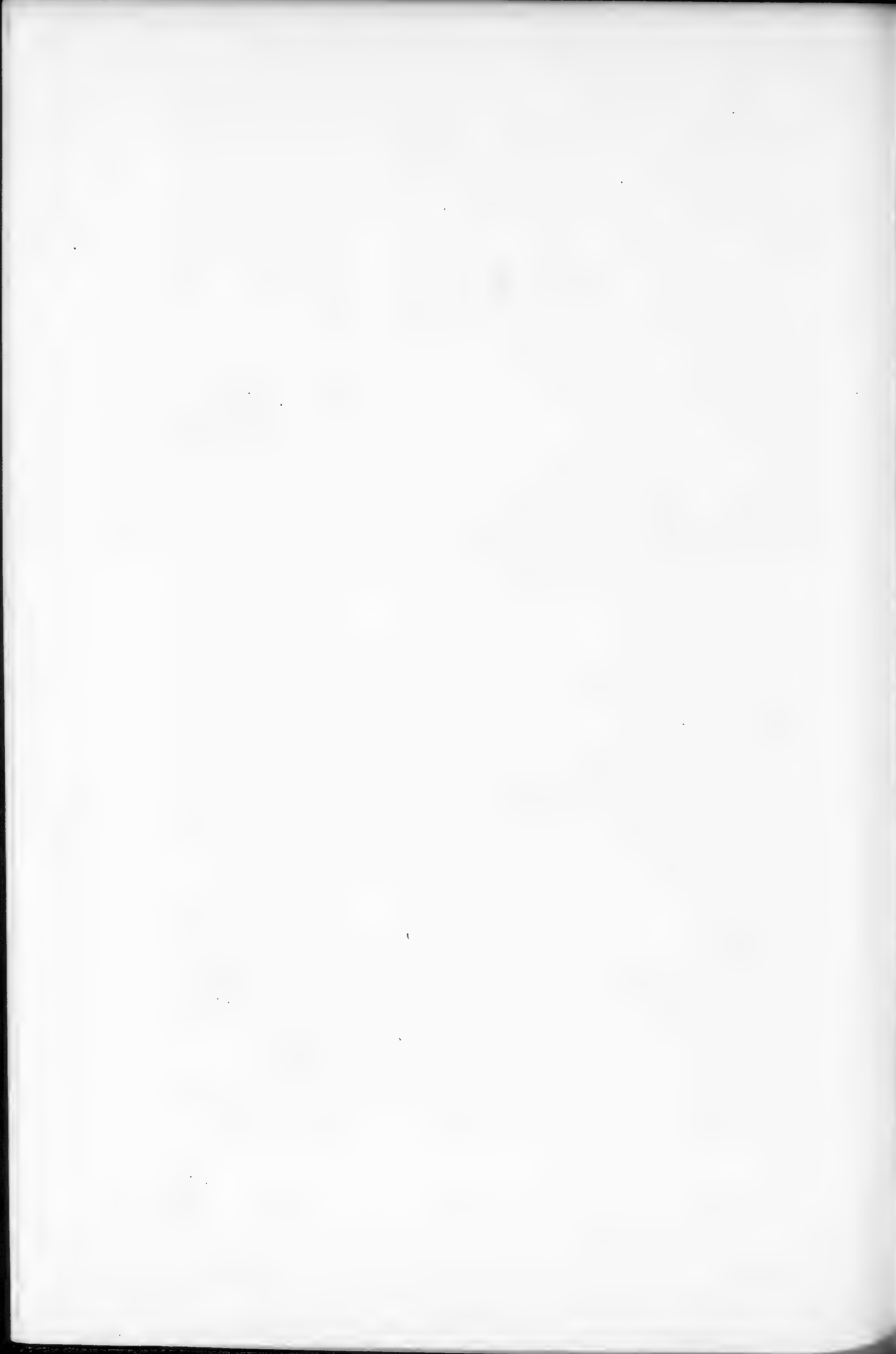
Fig. 112. The same, at another plane of focus, where the innermost structure is not brought out as in Fig. 111.



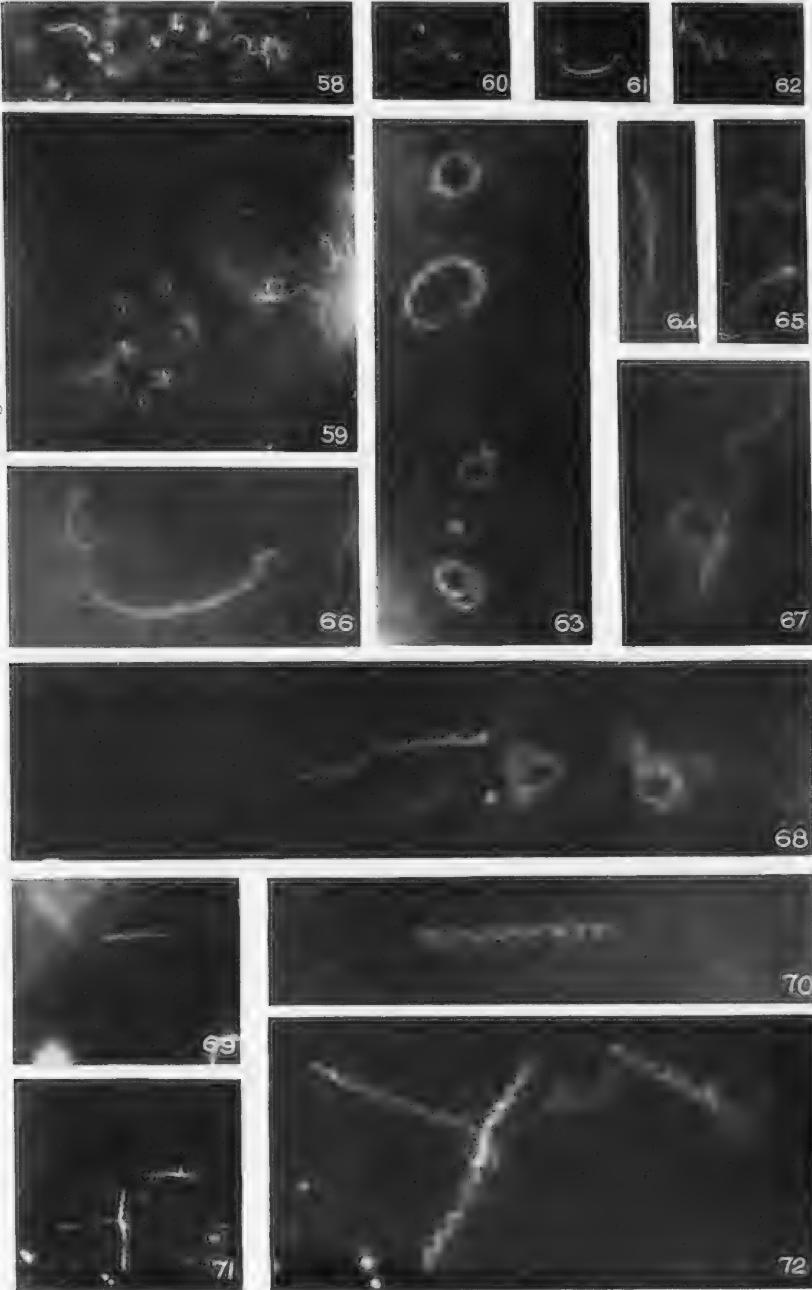
(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)



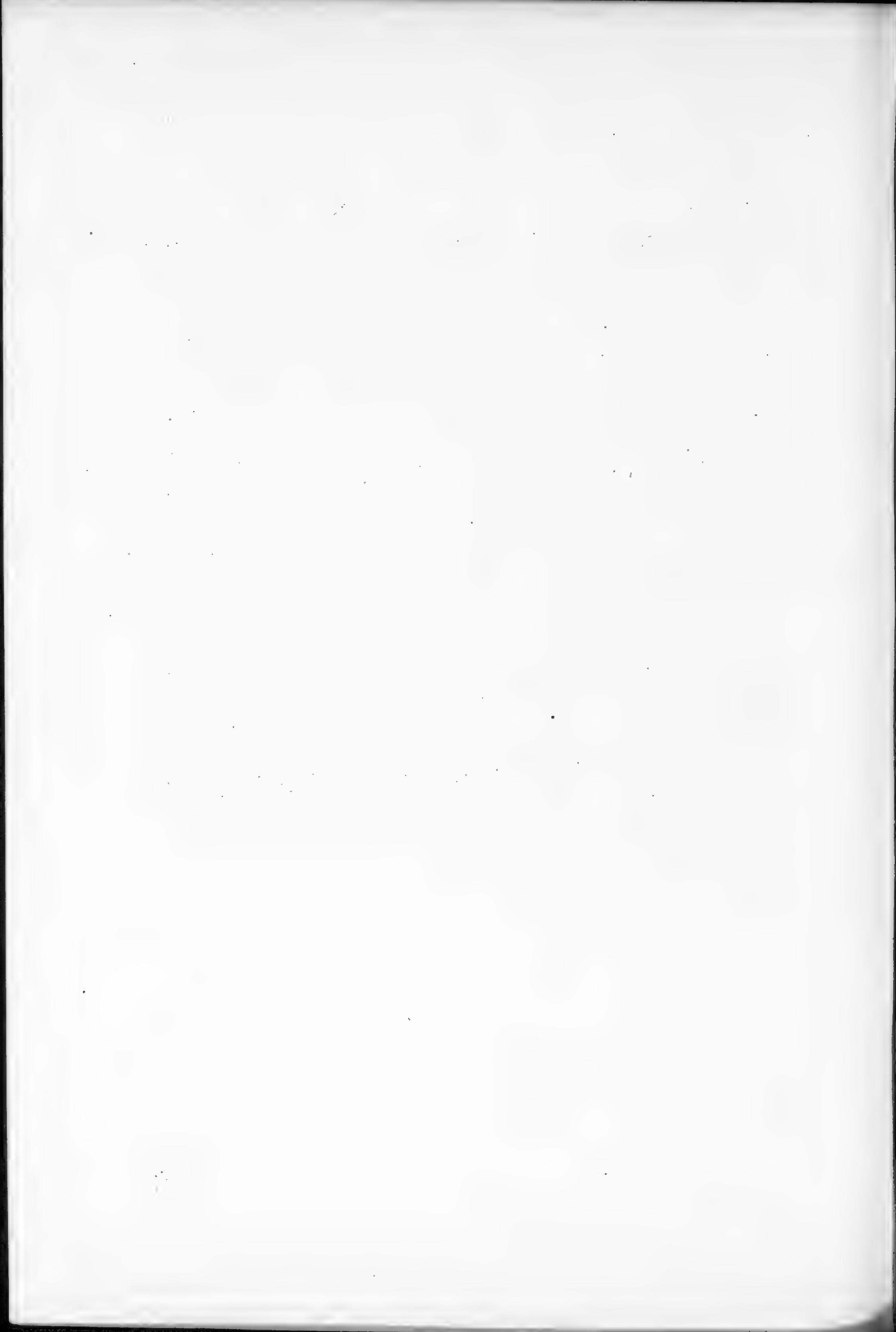
(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)

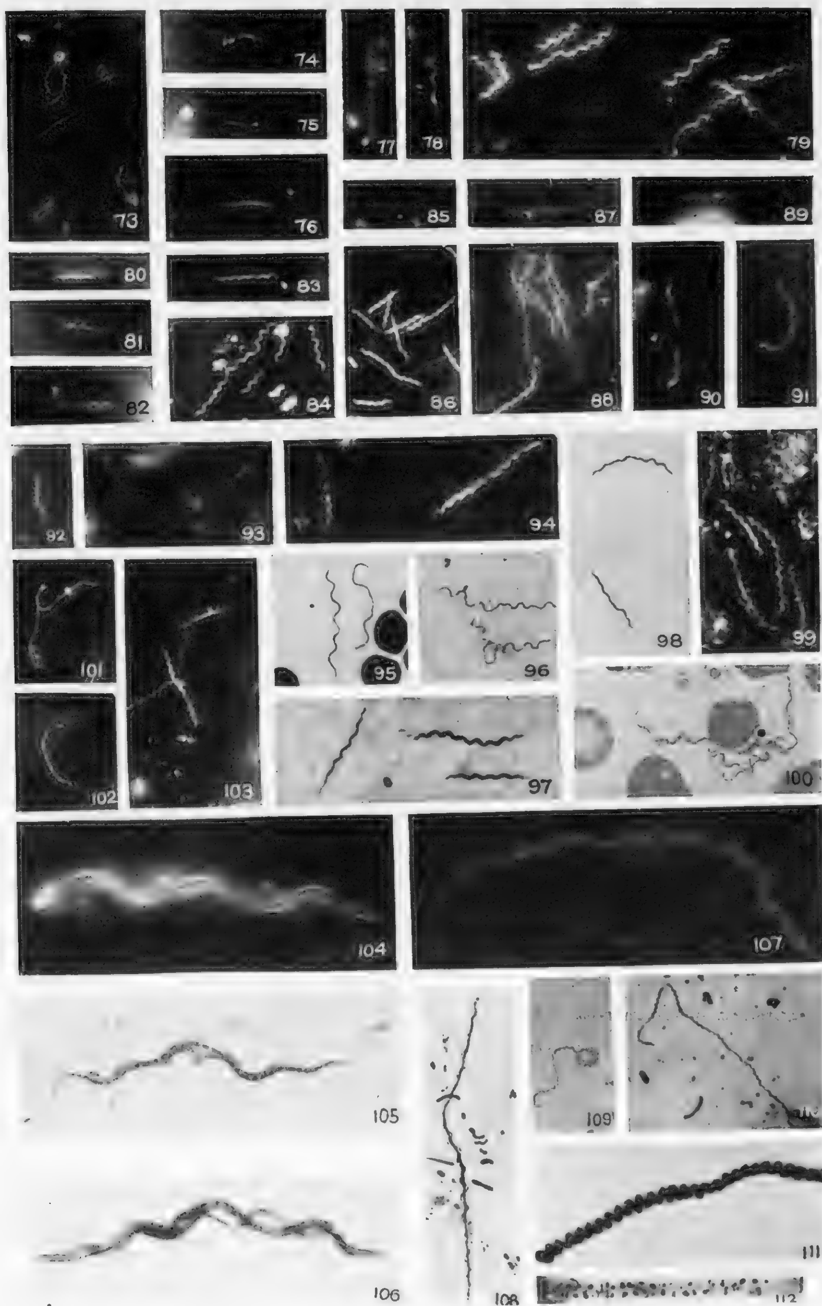


(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)

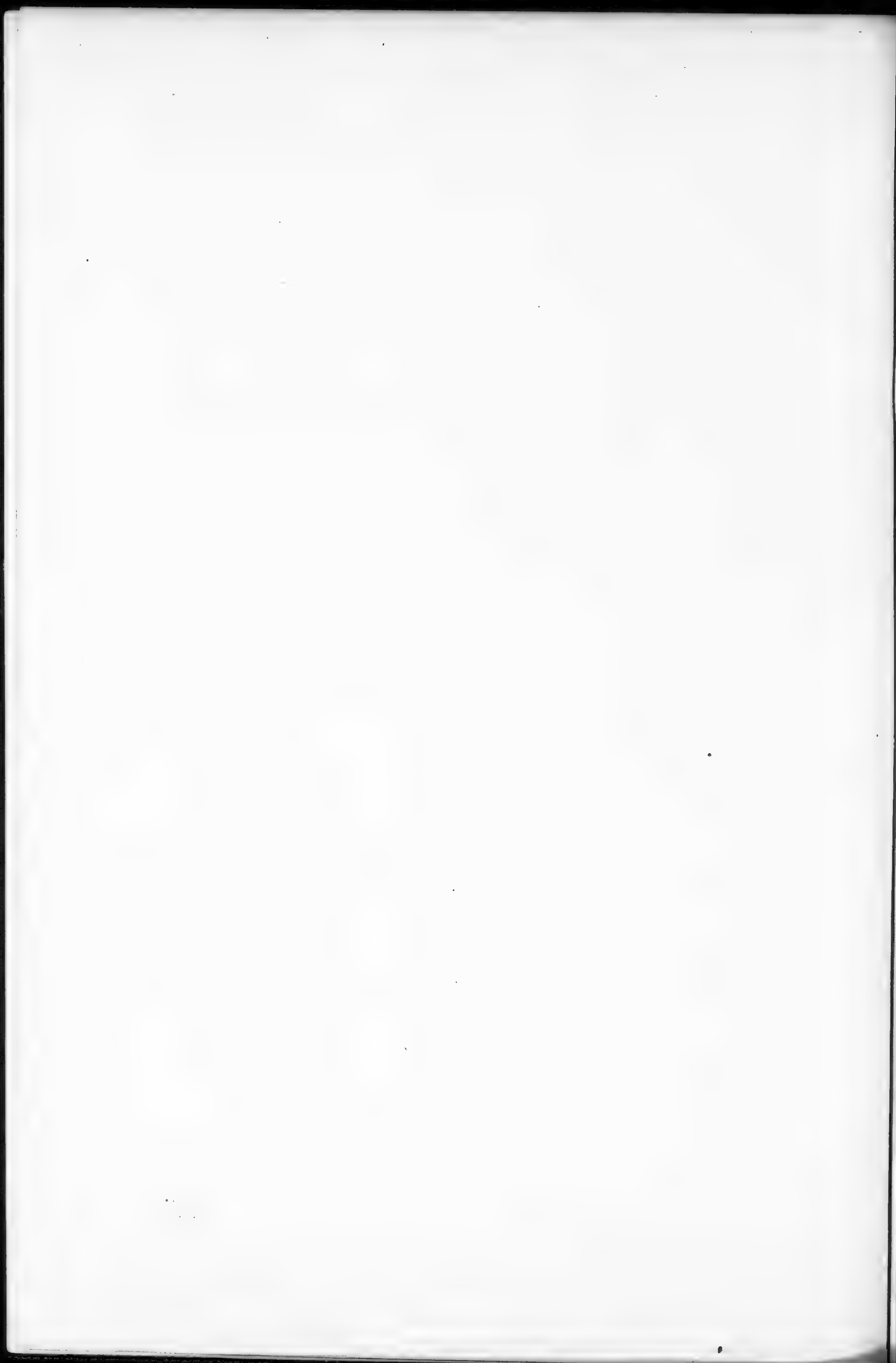


(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)





(Noguchi: Nomenclature of *Leptospira icterohæmorrhagiæ*.)



FURTHER STUDY ON THE CULTURAL CONDITIONS OF LEPTOSPIRA (SPIROCHÆTA) ICTEROHÆMORRHAGIÆ.

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The cultivation of *Leptospira* (*Spirochæta*) *icterohæmorrhagiæ*[1] is comparatively simple. It was first accomplished by Inada and his coworkers[2] by means of the method recommended by me for the cultivation of several varieties of blood spirochetes.[3] Later, various techniques for the isolation of this organism on artificial media were proposed by Ito and Matsuzaki,[4] Reiter,[5] Martin, Pettit, and Vaudremer,[6] and myself.[7] While all the methods appear to have given satisfactory results, there is no unanimity as to the best one to be followed in routine work. As far as I am aware, there has been no critical analysis of the conditions requisite for uniform success in obtaining a culture. I wish to report here some of the results of my study of the various strains from Asiatic, European, and American sources.

Necessity of Fresh Serum Constituents for the Growth of Leptospira icterohæmorrhagiæ.

My first cultures of the Japanese, European, and American strains of *Leptospira icterohæmorrhagiæ* were obtained by employing a medium containing about 1 part of normal rabbit serum and 2 parts of Ringer's solution, with the addition of an adequate amount of citrate plasma.[8] The rate of multiplication of the organism is faster at 37°C.

[1] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 575.

[2] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[3] Noguchi, *J. Exp. Med.*, 1912, xvi, 199.

[4] Ito, T., and Matsuzaki, H., *J. Exp. Med.*, 1916, xxiii, 557.

[5] Reiter, H., *Deutsch. med. Woch.*, 1916, xlii, 1282.

[6] Martin, L., Pettit, A., and Vaudremer, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 197.

[7] Noguchi, *J. Exp. Med.*, 1917, xxv, 755.

[8] About 0.5 part.

than at 25°C., but on the whole the first generation grows much more slowly than a later generation. It may be several days before growth is definitely ascertained.

The question may be raised as to what part of the serum is essential for the cultivation of the organism. For the purpose of determining this point, a portion of a mixture of rabbit serum 1 part, and Ringer's solution 3 parts, was heated to 60°C. for 30 minutes and another portion to 100°C. for 15 minutes. Unheated serum was used as control. It was found that heating to 100°C. for 15 minutes destroyed the nutrient value of the rabbit serum. Heating to 60°C. for 30 minutes reduced but did not destroy its cultural value as compared with the control. The nutrient principle of the serum, therefore, is closely associated with coagulable serum proteins. Filtration through the Berkefeld filter does not alter the cultural value of the serum medium.

Comparative Nutrient Value of Various Sera.

Not many animals are susceptible to the inoculation of *Leptospira icterohæmorrhagiæ*, and the guinea pig is the only animal in which the infection is almost invariably fatal. Rabbits are comparatively resistant, 1 to 2 cc. of a well growing pure culture being required to produce jaundice, whereas 0.000001 cc. of the same cultures may cause typical symptoms and death in a guinea pig. Dogs are more sensitive than rabbits, while cats, white rats, mice, and wild rats tolerate the infection and become carriers. A comparison of the suitability of various animal sera for purposes of cultivation of the organism is of practical as well as of biological interest.

Sheep Serum.—Of twelve different sheep sera, only four were found to be suitable, and in these the life of the organism was much shorter than in rabbit serum medium. A mixture of serum 1 part, Ringer's solution 3 parts, and 1.5 per cent agar 0.5 part was used. Undiluted sheep sera gave no better results, nor was the use of the citrate plasma from sheep advantageous.

Guinea Pig Serum.—Eight different lots of guinea pig sera were tested, each lot containing the sera from several animals, and good results were obtained in all. The sera were diluted three times with Ringer's solution and a small amount of agar or citrate plasma was

added. In this medium, however, the organism died out much sooner than in the rabbit serum medium.

Horse Serum.—Two out of four different horse sera proved to be very satisfactory, especially when used in a mixture of 1 part serum, 3 parts Ringer's solution, and 0.5 part 1.5 per cent agar. In this medium the culture survived for many weeks.

Calf Serum.—Only two calf sera were tested, but both gave a fairly good growth. A 1: 4 dilution of serum with Ringer's solution was better than the undiluted serum. Martin, Pettit, and Vaudremer[6] recommend a 1: 10 dilution of this serum as most suitable.

Goat Serum.—The only serum tested was very suitable when used in a mixture of 1 part serum, 3 parts Ringer's solution, and 0.5 part 1.5 per cent agar. The undiluted serum did not give so good a growth.

Donkey Serum.—The one available specimen proved totally unsuitable.

Pig Serum.—Two pig sera were tested, but the culture failed to grow in any concentration.

Rat Serum.—The sera from about twenty white rats were mixed and tested for their nutrient value. Diluted as well as undiluted sera were employed, but the results were negative.

Human Serum.—Five specimens which had been collected many months previous to the time of testing proved to be without any nutrient value for the organism in question. Two other specimens, which were freshly collected[9] from syphilitic patients, were found to be fairly suitable when used in proportions of 1: 1 and 1: 3 with Ringer's solution. The culture was short lived, however, reaching its greatest growth in about 11 days at 37°C. and dying off during the following week. The growth of the culture in the rabbit serum control medium was still increasing when the other cultures died.

Ascitic Fluid.—Twenty different samples of ascitic fluid were tested. They were used undiluted and also in different dilutions with Ringer's solution, but up to the present time none has been found suitable for the cultivation of *Leptospira icterohæmorrhagiæ*.

[9] These specimens were obtained through the courtesy of Dr. David J. Kaliski.

Nutrient Value of Organ Emulsions.

In the later stages of infection *Leptospira icterohæmorrhagiæ* invades the visceral organs in enormous numbers, the liver and kidneys being principally involved. One might infer, therefore, that these organs contain an abundant quantity of the substances favorable for the life and multiplication of the organism, and that an emulsion of these organs would constitute an ideal culture medium. The experimental data, however, did not support this assumption.

Emulsions of approximately 5 per cent in Ringer's solution were prepared with the liver, kidney, spleen, heart muscle, and testicle of a normal rabbit and a normal guinea pig, killed by bleeding, and tested for their nutrient value as culture media. In order to make the conditions of the media as varied as possible, the emulsions were used in four different ways: in one set of tubes the emulsion was used alone and unheated, in the second it was heated to 60°C., in the third it was heated to 100°C., and in the fourth there was added agar amounting to 0.3 per cent. The mixture of rabbit serum, Ringer's solution, and citrate plasma and that of rabbit serum, Ringer's solution, and agar were used as control media.[7] In the media containing the organ emulsions no sign of growth of the spirochete was observed, while excellent cultures were obtained in the control media.

The organs of guinea pigs were just as unsuitable for the cultivation of the organism as those of rabbits. I was not unaware of the possible change in the reaction due to autolysis of the organ cells, or of the injurious effect which certain autolytic cleavage products might have, but the emulsions showed a weak alkaline reaction throughout the experiments.

When rabbit serum, in the proportion of approximately 25 per cent, was added to a number of the tubes containing the emulsions, the spirochete multiplied vigorously; therefore, the fact that no culture was obtained with pure organ emulsions must have been due to the absence of suitable nutrient substances for the organism.

Egg White and Egg Yolk as Culture Media.

The failure of various organ emulsions to serve as culture media turned my attention to the possibility of utilizing egg white and egg

yolk for the purpose. The white and yolk of an egg were separated and each was diluted with Ringer's solution in different proportions: 2.5 cc. + Ringer's solution 2.5 cc.; 1 cc. + Ringer's solution 4 cc.; 0.5 cc. + Ringer's solution 4.5 cc.; and 0.25 cc. + Ringer's solution 4.75 cc. In each instance one set of tubes was used in the fresh state and the other heated to 55°C. for 24 hours with a view to possible improvement of nutrient value. In none of the egg media was any culture obtained, nor did the addition of the rabbit serum enhance their nutrient value beyond that of the serum.

Concentration of the Serum in Culture Media.

The importance of the presence of serum for the successful growth of the spirochete having been demonstrated, the following experiments were undertaken in order to determine the influence of various

TABLE I.

Japanese strain.	37°C.		26°C.	
	7 days.	30 days.	7 days.	30 days.
Undiluted rabbit serum	+	+++	+	+++
50 per cent rabbit serum + Ringer's solution	+	+++	+	+++
33 " " " " + " "	+	+++	+	+++
25 " " " " + " "	+	+++	+	+++
20 " " " " + " "	+	+++	+	+++
15 " " " " + " "	+	+++	+	+++
10 " " " " + " "	+	+++	++	+++
5 " " " " + " "	+	+	+	+++

European strain.	Cultures, 30 days at 26°C.	Proteins precipitable with 10 volumes of absolute alcohol.
33 per cent rabbit serum + Ringer's solution	+++	Copious coarse precipitate and opalescence.
20 " " " " + " "	++	Copious coarse precipitate and opalescence.
10 " " " " + " "	++	Minute granules and opalescence.
5 " " " " + " "	+	Opalescence.
2 " " " " + " "	−	"
1 " " " " + " "	−	Granular.
0.5 " " " " + " "	−	

TABLE I—*Concluded.*

American strains.	Cultures, 30 days at 26°C.		
	Strain 1	Strain 2	Strain 3
33 per cent rabbit serum + Ringer's solution...	+++	+++	+++
20 " " " " + " " ...	+++	+++	+++
10 " " " " + " " ...	++	+++	++
5 " " " " + " " ...	+	++	+
2 " " " " + " " ...	−	−	−
1 " " " " + " " ...	−	−	−
0.5 " " " " + " " ...	−	−	−

The above experiments show that a maximum growth may be obtained with all strains tested in a medium containing more than 20 per cent serum, while a 10 per cent serum medium may give as much growth, but only with certain strains. The growth is scanty in a 5 per cent serum solution, and in a medium containing 2 per cent or less there is no growth.

concentrations of serum upon the culture. Table I summarizes the results.

Influence of Diluents and of Salt Concentration upon the Culture.

The apparent indifference of the spirochete to salt constituents of the culture media was noticed from the beginning of the cultivation

TABLE II.

American strain No. 1	Cultures 30 days at 26° C.
Rabbit serum 1 cc. + 10 per cent sodium chloride 4 cc. = 8 per cent sodium chloride	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 2 cc. + water 2 cc. = 4 per cent sodium chloride	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 1 cc. + water 3 cc. = 2 per cent sodium chloride	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 0.5 cc. + water 3.5 cc. = 1 per cent sodium chloride	+++
Rabbit serum 1 cc. + 10 per cent sodium chloride 0.25 cc. + water 3.75 cc. = 0.5 per cent sodium chloride	+++
Rabbit serum 1 cc. + water 4 cc. = salt-free control	+++
" " 1 " + Ringer's solution 4 cc. = serum-Ringer's solution control	+++

experiments. Instead of Ringer's solution, a 0.9 per cent saline solution or distilled water could be used as a diluent. In fact sewer water and stagnant or ordinary tap water were found to be satisfactory diluents when previously rendered sterile by filtration or autoclaving. The organism displays great tolerance not only to various neutral salts or organic matter which are apt to be present in sewer or stagnant water, but also to an increasing concentration of sodium chloride. The relation of salt concentration to growth is shown in Table II.

There was no perceptible difference in the degree of growth of the organism in this experiment, or in its morphological features. The tonicity of the culture medium is apparently an unimportant factor.

Effect of Reaction upon the Culture.

Leptospira icterohæmorrhagiæ seems to be one of the most sensitive of the microorganisms to the reaction of the culture medium. A slight variation to acid or alkaline from a given optimum zone renders a medium totally unsuitable for the growth of the organism (Table III).

Considering the minuteness of the quantities of hydrochloric acid or sodium hydroxide which were added in these experiments, and the extent to which the reagents were finally diluted with serum and distilled water, one cannot fail to realize the great importance which the reaction of the culture medium must have in relation to the growth of the organism. Similar results were obtained with the Japanese and European strains. The first requisite to the successful cultivation of *Leptospira icterohæmorrhagiæ* appears to be an optimum reaction of the culture medium, which, in my experience, lies between a slight alkaline reaction and that resulting from subsequent multiple dilutions with indifferent diluents (distilled water, isotonic salt solution, Ringer's solution, etc.).

A considerable fluctuation was found by titration of the sera of several domestic animals. For example, 2 cc. of the sera of the sheep, donkey, ox, and pig, each mixed with 3 cc. of distilled water, required 0.4 cc. of 0.1 N hydrochloric acid to bring about a neutral reaction, and 0.6 cc. to cause distinct acidity and turbidity. Rabbit serum had a uniformly weaker reaction, only 0.2 cc. of 0.1 N hydro-

TABLE III.

American strain No. 1	Physical changes.	Reaction to litmus paper.	Result of cultivation at 30° C. for.	
			6 days.	18 days.
Rabbit serum 1 cc. + water 3 cc.	Clear.	Slight alkaline.	+++	++
" " 1 " + 1.5 per cent agar 0.5 cc.	"	Slight alkaline.	+++	++++
Addition of acid.				
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.1 cc.	Slight opalescence.	Neutral.	+	–
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.1 cc. + 1.5 per cent agar 0.5 cc.	Slight opalescence.	"	+	–
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.2 cc.	Many sandy precipitates on wall and bottom.	Trace of acid.	–	–
Rabbit serum 1 cc. + 0.1 N hydrochloric acid 0.2 cc. + 1.5 per cent agar 0.5 cc.	Slight opalescence.	Trace of acid.	+	–
Addition of alkali.				
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.1 cc.	Clear.	Distinct alkaline.	–	–
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.1 cc. + 1.5 per cent agar 0.5 cc.	"	Distinct alkaline.	–	–
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.2 cc.	"	Stronger alkaline.	–	–
Rabbit serum 1 cc. + 0.1 N sodium hydroxide 0.2 cc. + 1.5 per cent agar 0.5 cc.	"	Stronger alkaline.	–	–

chloric acid being required to produce a neutral, and 0.35 cc. an acid reaction. The reaction of horse serum lay between that of the rabbit and that of the other animals. The fact that some of the latter sera showed a better nutrient value in dilution may be explained by the reduction of native alkalinity through dilution.

Oxygen Requirement of the Culture.

At the beginning of these cultivation experiments, I supposed *Leptospira icterohæmorrhagiæ* to be an obligatory or facultative anaerobe, because of its great facility for invading organs and multiplying in them. All attempts at cultivation failed as long as cultural conditions were employed which were calculated to produce anaerobiosis. The combination of conditions which I designated as aerotropic anaerobiosis[10] several years ago, and which was successfully used for the cultivation of the relapsing fever spirochetes, gave fairly good results when a suitable serum was used. But in the tubes to which a piece of fresh rabbit kidney was added, the cultures grew less luxuriantly and died out sooner than in the control tubes without the tissue. The simplicity of the cultural requirements of this organism was a surprise and led to the inference that the organism is an aerobe. When a number of subcultures of the Japanese, European, and American strains were cultivated at 37°C. in an anaerobic apparatus and another set without the exclusion of oxygen, excellent growth took place in all tubes where oxygen was accessible, while not a single organism could be found in the tubes kept in an anaerobic apparatus. The tubes were taken out of the anaerobic jar after 12 days and allowed to stand for several days at 37°C., but no new culture developed, probably because of the death of the organism during its stay in the anaerobic apparatus. *Leptospira icterohæmorrhagiæ*, therefore, has been shown to be an obligatory aerobe.

Detrimental Conditions Caused by Physical Hindrances to the Penetration of Oxygen into the Medium.

For obligatory aerobic bacteria a slant or plate agar or broth should be satisfactory, because most of this class of organisms grow in more

[10]Noguchi, *The Harvey Lectures*, 1915–16, 236.

or less discrete, often thick or elevated colonies on the surface of a solid medium. In broth the growth may be diffuse or superficial, forming a pellicle or thick scum. The use of a high layer agar or gelatin for the cultivation of such organisms means a waste of medium, since oxygen cannot penetrate the greater part of it. Since *Leptospira icterohæmorrhagiæ* is an obligatory aerobe, it follows that the addition of solid substances such as agar or gelatin, which must necessarily interfere with the entrance of oxygen into the medium, will be detrimental to the growth of the organism. The denser the concentration of agar or gelatin, the narrower is the zone to which oxygen can penetrate. The experiment summarized in Table IV shows the effect of different concentrations of agar or gelatin upon the culture. The gelatin and agar were made in a 0.5 per cent saline solution and adjusted to a slightly alkaline reaction.

TABLE IV.

Medium.	37°C.			26°C.		
	4 days.	7 days.	28 days.	4 days.	7 days.	28 days.
Gelatin (10 per cent) 4 cc.	−	−	−	−	−	−
Rabbit serum 1 cc. Gelatin (10 per cent) 3 cc.	+	−	−	<<+	−	−
Rabbit serum 1 cc. Gelatin (10 per cent) 1 cc. Ringer's solution 2 cc.	+	+++	+++	<+	+	+++
Rabbit serum 1 cc. Gelatin (10 per cent) 0.5 cc. Ringer's solution 2.5 cc.	+	+++	+++	<+	<+	+++
Agar (2 per cent) 4 cc.	−	−	−	−	−	−
Rabbit serum 1 cc. Agar (2 per cent) 3 cc.	+	++	<+	<<+	+	<<+
Rabbit serum 1 cc. Agar (2 per cent) 1 cc. Ringer's solution 2 "	+	++++	++++	+	++	++++
Rabbit serum 1 cc. Agar (2 per cent) 0.5 cc. Ringer's solution 2.5 "	++	++++	++++	+	++	++++

The experiment demonstrates the disturbing effect of gelatin when present in more than 7.5 per cent and of agar in more than 1.5 per cent. Agar, when added in proportions of 0.5 per cent and 0.25 per cent, considerably improved the cultural conditions. In this concentration it does not perceptibly hinder the penetration of oxygen into the medium and it offers to the spirochetes an ideal semisolid permeable substance. In this respect this particular culture medium is even better than a pure fluid medium. Gelatin, when added in proportions of 2.5 and 1.25 per cent, seems to have been neither beneficial nor detrimental to the growth of the culture.

Ordinary Culture Media and Leptospira icterohæmorrhagiæ.

It would be an economic advantage if a simpler method for the cultivation of this spirochete was devised. No culture was obtained, however, with any of the ordinary media, such as plain and 2 per cent glucose bouillon, Hiss serum water, litmus milk, plain and 2 per cent glucose agar, Loeffler's serum, glycerolated bouillon, and agar. A special bouillon medium formulated by Dr. Kligler was tried—1 per cent peptone, 0.5 per cent sodium phosphate, 0.1 per cent glucose, and 0.5 per cent sodium chloride—but without success. The presence of peptone, broth, casein, glucose, etc., instead of having a nutrient value for *Leptospira icterohæmorrhagiæ* in a suitable medium such as one containing the necessary amount of rabbit serum, seems to have a definite unfavorable influence upon the culture. The addition of a 10 per cent neutral solution of peptone 4.5 cc., to rabbit serum 1.5 cc., rendered the mixture unsuitable for a culture medium, as is not the case with indifferent diluents such as Ringer's solution, distilled water, or isotonic salt solution. Even the addition of approximately 1.5 per cent peptone suppressed growth to a marked degree. Bouillon or glucose bouillon are not good diluents for making up a culture medium for this organism.

Addition of Carbohydrates to Culture Media.

Akatsu,[11] while working in my laboratory, studied the action of various spirochetes upon many carbohydrates, but he did not find

[11] Akatsu, S., *J. Exp. Med.*, 1917, xxv, 375

definite fermentation phenomena in any of the organisms examined. With *Treponema mucosum* and *Treponema microdentium* a definite increase in the amount of acid was noticed. In the present experiment, the Japanese strain of *Leptospira icterohæmorrhagiæ* was cultivated in two sets of media of fourteen tubes each. In one set the media were made up of 1.5 cc. of rabbit serum, 1 cc. of a 10 per cent solution of carbohydrate, previously sterilized by filtration, 2.5 cc. of Ringer's solution, and 1 cc. of citrate plasma of the rabbit. In the other set 1 cc. of 2 per cent agar (melted) was used instead of the citrate plasma. The fourteen carbohydrates used in both sets were glucose, lactose, maltose, levulose, galactose, saccharose, dextrin, inulin, mannite, dulcite, isodulcite, arabinose, raffinose, and salicin. Tubes without carbohydrate and tubes also which were not inoculated with culture were used as controls.

The new generation of the culture became recognizable within a fortnight at 29°C. by the hazy layer at the top of the columns of culture media. The haze extended downwards from the surface to a depth of 1 to 1.5 cm. By examination under the dark-field microscope, the haze was found to represent dense diffuse colonies of actively multiplying spirochetes. The appearance of the haze was the same in the tubes containing the various carbohydrates as in the sugar-free control tubes. In the set where 1 per cent citrate plasma was used to form loose fibrin, the haze was less distinct but extended as far as 3 or 4 cm. below the surface, and the lower border was not sharply outlined as in the media with semisolid agar. The viability of the spirochetes was as great in the media containing carbohydrates as in those without carbohydrates. The reaction of the cultures failed to indicate any attack by the organism upon the carbohydrates. The reaction remained slightly alkaline to litmus paper as before cultivation, and was entirely comparable with the reaction in the spirochete-free controls.

Special attention was given to the detection of possible morphological modifications in the organisms grown in the presence of the carbohydrates, but none was recognized.

Influence of Temperature upon Cultivation.

Inada and his coworkers[2] found that *Leptospira icterohæmorrhagiæ* grows very well at room temperature, as it does at any temperature up to 37°C., but that at lower temperatures (20–25°C.) the organism survives longer than at 37°C.

I have cultivated three different strains of the spirochete at different temperatures. The results, as recorded in Table V, are self-explanatory. The media used consisted of rabbit serum 1 cc. + Ringer's solution 3 cc. + citrate plasma 1 cc. or 1.5 per cent agar 1 cc.

TABLE V.

Strains.	42°C.		37°C.		30°C.		25°C.		10°C.	
	7 days.	28 days.	7 days.	28 days.	7 days.	28 days.	7 days.	28 days.	7 days.	28 days.
Japanese.										
Plasma.	–	–	++++	++++	++++	++++	++++	++	++++	++
Agar.	–	–	+++	++++	+++	++++	+	<+	+	<+
European.										
Plasma.	–	–	++	+++	++	++	+	–	+	–
Agar.	–	–	++	+++	++++	++++	+	++	<<+	–
American No. 1.										
Plasma.	–	–	++	+++	++	+++	+	++	+++	++
Agar.	–	–	++	+++	++	++++	++	+++	++	+

The ability of the organism to multiply and remain active a long time at 10°C. is interesting from the epidemiological standpoint. It suggests that certain insects might serve as reservoirs of the virus.

Culture Media Recommended for Leptospira icterohæmorrhagiæ.

As a result of the experiments recorded on the relative nutrient value of various sera, the influence of reaction, oxygen tension, diluents, salts, and various other substances, I have formulated the following media:

A.	Rabbit serum	1.5 parts.	
	Ringer's solution	4.5 "	
	Citrate plasma	1.0 part.	
	Paraffin oil to cover the surface.		
B.	Rabbit serum	1.5 parts.	
	Ringer's solution	4.5 "	
	2 per cent agar	1.0 part.	
	Paraffin oil to cover the surface.		
C.	Rabbit serum	1.5 parts.	Semisolid portion.
	Ringer's solution	4.5 "	
	2 per cent agar	1.0 part.	
	After solidification add:		
	Rabbit serum	1.5 parts.	Fluid portion.
	Ringer's solution	4.5 "	
	Paraffin oil to cover the surface.		

Growth usually begins much sooner in Medium A than in Medium B, but after a month more spirochetes will be found in B. For keeping up subcultures of various strains, Media A and B were simultaneously used in small test-tubes each containing 7 cc. of the composite medium.

For obtaining a large amount of culture, long necked flasks of medium capacity (50 to 100 cc.) were used. It was found best to fill the flasks with the medium to one-half or one-third their capacity and then to cover the surface with a very thin layer of paraffin oil. If the flasks are filled higher than this, oxygen becomes less accessible to the deeper part of the medium, especially when it contains agar. The use of a low layer semifluid medium (B) is based upon the fact, previously mentioned, that unrestricted multiplication of *Leptospira icterohæmorrhagiæ* takes place in such a medium on the surface stratum of 1 to 2 cm. Medium A is similarly semifluid, but the fibrin mass loosens and breaks up in time, especially by repeated withdrawal of the culture with pipettes, rendering the penetration of oxygen almost as easy as in a fluid medium. The flasks containing Medium A may therefore be filled half or two-thirds full, with a thin layer of paraffin oil.

Medium C seems to combine the advantages of Media A and B, the lower stratum being composed of Medium B, upon which, after solidification, is superimposed a mixture of rabbit serum and Ringer's solution (1:3). The medium is then inoculated and covered with a thin layer of paraffin oil. For subcultures, 0.1 or 0.2 cc. of a vig-

orously growing culture is pipetted on the surface of new culture media and then covered with paraffin oil.

D is a medium for acclimated strains. A fluid medium consisting of 1 part of horse or sheep serum and 3 parts of Ringer's solution or salt solution proved to be fairly suitable for strains which had become accustomed to the various media (A, B, C) during a period of several months.

SUMMARY AND CONCLUSIONS.

1. The presence of suitable animal or human serum is essential for the cultivation of *Leptospira icterohæmorrhagiæ*.

2. The nutrient value of serum is considerably reduced by heating to 60°C. for 30 minutes and is destroyed by boiling (100°C). Filtration through a Berkefeld filter does not diminish the nutrient value of the serum.

3. The cultural value of different animal sera varies considerably. It is entirely absent from the sera of the rat and the pig. The sera of the rabbit, horse, and goat are better suited for the growth of the organism than those of the guinea pig, sheep, donkey, or calf. Human serum is suitable, but not ascitic fluid.

4. Fresh or heated emulsions of the liver, kidney, heart muscle, or testicle of the normal guinea pig or rabbit have no cultural value for the organism. The same may be said of both the white and yolk of the hen's egg.

5. A luxuriant growth takes place in a medium of Ringer's solution to which more than 10 per cent of normal rabbit serum is added. There is only moderate growth with 5 per cent of serum, and none when less than 2 per cent is present. The use of an undiluted serum offers no advantage over a diluted one, provided the latter contains at least 10 per cent of serum. In the case of certain animal sera dilution seems to make them more suitable for cultivation purposes, owing perhaps to its reduction of their inherent alkalinity.

6. The tonicity of the culture medium has but little influence upon the growth and morphology of the organism. A medium containing distilled water as diluent or one containing 8 per cent sodium chloride seems to give identical results. The viability of the organism was

greatest in a medium in which Ringer's solution or isotonic salt solution was used as diluent.

7. The reaction of the medium is an important factor in the cultivation of the organism, which thrives most vigorously in a medium of which the reaction is slightly alkaline, not exceeding that of the serum. If the reaction is neutral, the growth is meager, and the culture is short lived. When the reaction of a medium becomes alkaline by the addition of a small amount of sodium hydroxide, or faintly acid by the addition of a little hydrochloric acid, no growth can take place.

8. *Leptospira icterohæmorrhagiæ* is an obligatory aerobe. Any hindrance to the access of oxygen constitutes an unfavorable factor in obtaining a culture.

9. The addition of carbohydrates to media has no perceptible effect upon the growth or morphology of the organism. The reaction of the media is not modified by their presence.

10. *Leptospira icterohæmorrhagiæ* grows at any temperature between 37° and 10°C., the optimum zone being 30–37°C. Growth proceeds more rapidly at 37°C. than at 30° or at 25°, but the cultures remain viable much longer at the latter temperatures. No growth takes place at 42°C.

11. Three different media are described for the cultivation of freshly isolated strains. After prolonged cultivation on these media a strain may be readily cultivated in a serum diluted with Ringer's or isotonic salt solution.

THE SURVIVAL OF LEPTOSPIRA (SPIROCHÆTA) ICTEROHÆMORRHAGIÆ IN NATURE; OBSERVATIONS CONCERNING MICROCHEMICAL REACTIONS AND INTERMEDIARY HOSTS.

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A solution of the question of the survival of *Leptospira icterohæmorrhagiæ* in nature may be brought about (1) by following up directly the actual conditions to which the spirochetes cast off by the hosts or artificially mixed with urine or feces will have to submit, or (2) by mixing the spirochetes with each in turn of the various bacteria commonly encountered in feces, sewage, or soil, and then determining the results of their simultaneous existence in the same media. As I shall show, the spirochete of infectious jaundice is a very delicate organism and is rapidly overwhelmed by most of the bacteria from intestinal contents, sewage, or soil.

Urine in Relation to Leptospira icterohæmorrhagiæ.

The effect of urine upon the viability of *Leptospira icterohæmorrhagiæ* is of practical importance, since it has been found by previous investigators[1, 2, 3] that the urine of about 77 per cent of the patients recovering from infectious jaundice still contains the spirochete after a period of 2 to 5 weeks. They made the interesting observation that two-thirds of the positive urines, some containing numerous spirochetes, failed to produce the infection in guinea pigs. Since the urines came from cases of 15 days' standing or longer, the fact may be ex-

[1] Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1917, xxvi, 341.
[2] Garnier, M., and Reilly, J., *Compt. rend. Soc. biol.*, 1917, lxxx, 38.
[3] Cappellani, S., and Frugoni, C., *Sperimentale*, 1917, lxxi, 335.

plained by an attenuation in virulence of the organism during the course of the disease. I have seen one case in which the urine was still infective for guinea pigs 1 month after the onset of the disease.

In one series of experiments a sample of urine freshly collected from a healthy individual, who had no history of ever having had jaundice, was tested for its action upon the spirochetes. 10 cc. amounts of

TABLE I.

Leptospira icterohæmorrhagiæ introduced into.	Changes in appearance and reaction.	0.2 cc. of a well growing culture of American strain No. 1 inoculated into tubes containing.				
		Fluid indicated 6 cc.		Fluid indicated 3.5 cc. + rabbit serum 1.5 cc. + citrate plasma 1 cc.		
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	3 days.
Normal urine	Clear, strongly acid.	–	–	+	+	<+
Urine 10 cc. + 0.1 N sodium hydroxide 0.1 cc.	Clear, neutral.	<+	–	++	++	+
Urine 10 cc. + 0.1 N sodium hydroxide 0.2 cc.	Precipitate +, slightly alkaline.	<+	–	++	++	+
Urine 10 cc. + 0.1 N sodium hydroxide 0 4 cc.	Precipitate ++, moderately alkaline.	<+	–	+	+	<+
Urine 10 cc. + 0.1 N sodium hydroxide 0.8 cc.	Precipitate +++, markedly alkaline.	–	–	–	–	–

the urine, which was strongly acid and had a titer such that 10 cc. of it required 7 cc. of 0.1 N sodium hydroxide to become moderately alkaline, were measured into a number of test-tubes, to each of which was added normal sodium hydroxide solution, the quantities added varying in each case in order to obtain a series of reactions from the original acidity of the specimen to a markedly alkaline reaction.

Cultures were set up in two parallel series, using in each series the original and partially neutralized portions of the urine, but adding to one series suitable amounts of rabbit serum and citrate plasma. Table I summarizes the results.

As the table shows, the jaundice spirochetes survived at least 24 hours in the portion of urine to which a quantity of from 0.1 to 0.4 cc. of normal sodium hydroxide solution had been added, but no trace of them could be found in the original urine or in that receiving 0.8 cc. of the alkali. In plain Ringer's solution alone the organism lived 24 hours under similar conditions. After 48 hours there were no spirochetes in any of the tubes of the first series.

The results obtained with the urine containing rabbit serum and citrate plasma were different from those of the other series. There was a good growth in all the tubes containing the unalkalized urine, and also in those to which had been added from 0.1 to 0.4 cc. of normal sodium hydroxide solution. The growth was better and lasted longer in the tubes in which the urine showed a neutral or slightly alkaline reaction than in the unmodified or more strongly alkalized urines. There was no growth in the tube to which 0.8 cc. of the normal sodium hydroxide had been added. While there was unmistakable growth in the urine media with rabbit serum and citrate plasma, the organisms were viable for only 1 week at the longest. The presence of the urine apparently reduces very much the nutrient value of the rabbit serum and citrate plasma, as is shown by the fact that the use of Ringer's solution instead of urine enables the spirochetes to multiply progressively for at least 3 weeks. Not only is the urine devoid of cultural value for the organism, but its presence in an otherwise suitable medium renders the latter less suitable for the growth of the organism.

Feces in Relation to Leptospira icterohæmorrhagiæ.

The escape in feces of living *Leptospira icterohæmorrhagiæ* from experimentally infected guinea pigs seems to be rather frequent. Ido and his coworkers,[1] for example, succeeded in producing typical spirochetosis in seven out of eleven animals tested with a corresponding number of specimens of feces; yet in spite of this high percentage of

TABLE II.

Specimens of feces from which media were prepared.	1:10 dilution.		1:10 dilution, autoclaved.		1:100 dilution, filtrate.	
	No blood.	Blood and serum added.	No blood.	Blood and serum added.	No blood.	Blood and serum added.
Normal feces No. 1	24 hrs.: A few distorted spirochetes among innumerable bacteria. No trace of the spirochetes after 48 hrs. Four daily inoculations (scarified skin) in guinea pigs all negative.	Same as preceding.	A few motile spirochetes after 24 hrs. but none after 48 hrs. No difference between normal and jaundice feces. Four daily inoculations (intraperitoneal) in guinea pigs all negative.	Moderate multiplication of spirochetes in 4 days, which gradually increased. Four daily inoculations (intraperitoneal) all positive.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days. Three daily inoculations all positive.
" " " 2				Moderate multiplication of spirochetes in 4 days, which gradually increased. No animal inoculations.	Spirochetes survived 4 days, after which they disappeared. No animal inoculations.	A good growth in 4 days which progressed well for 10 days. No animal inoculations.
Jaundice feces No. 1				Moderate multiplication of spirochetes in 4 days, which gradually increased.	Spirochetes survived 4 days, after which they disappeared.	A good growth in 4 days which progressed well for 10 days. Three daily animal

						inoculations. All positive.
Jaundice feces No. 2				Four daily inoculations. All positive. Moderate multiplication of spirochetes in 4 days, which gradually increased. No animal inoculations.	One 1st day inoculation positive. Spirochetes survived 4 days, after which they disappeared. No animal inoculations.	A good growth in 4 days which progressed well for 10 days. No animal inoculations.

positive results, it was by no means easy to demonstrate the presence of the spirochetes in feces with the dark-field microscope, the organism being found only once in the stools from 60 guinea pigs having spirochetosis. The specimens of feces which were infective usually contained erythrocytes. In human cases one specimen of feces out of seven was found to be infective.

There is apparently a possibility, then, that the spirochetes are excreted from patients in the feces. To determine the viability of the spirochetes under these conditions, a series of experiments was performed in which they were added in large quantities to feces, both with and without a simultaneous addition of blood ingredients. Two specimens of feces from normal individuals were used, and two from cases of jaundice in children, with the characteristic clay color. Each specimen was used in three ways: (1) as a moderately thick emulsion (1:10) in Ringer's solution, without sterilization; (2) the same sterilized by autoclave; (3) a Berkefeld V filtrate of a dilute fecal emulsion (1:100). Two sets of tubes were set up, each containing 5 cc. of one of the variously prepared suspensions. To each of the tubes of one set were added a few drops of defibrinated blood and 1 cc. of serum from a normal rabbit, and to all the tubes were added 2 cc. of a richly growing culture of either the American or the European strain of the organism. Both sets of tubes were then placed at a temperature of 26°C.

The fate of the spirochetes under these conditions was followed daily by direct microscopic examination and indirectly by inoculation tests on guinea pigs. Table II summarizes the results obtained with the European strain. The results with the American strain were practically identical and are consequently not recorded here.

As is apparent from the table, the spirochete cannot survive in a fecal emulsion, even when there are present sufficient nutrient elements, longer than 24 hours at a temperature of 26°C. That this fact is due to the simultaneous presence of various bacteria, which rapidly overgrow the delicate spirochetes and deprive them of the necessary nutrient substances, is inferred from the much longer survival of the spirochetes in the tubes containing the sterilized emulsion, particularly in those to which were added the blood and serum. In the latter tubes, in fact, there was a temporary multiplication of the

organisms lasting several days. In the tubes containing a dilute, sterile, fecal filtrate, the spirochetes survived at least 4 days, and the addition of blood and serum caused the filtrate to become a suitable culture medium, if not equally as good as Ringer's or saline solution. Inoculations made with the mixtures of the non-sterilized fecal emulsions, with and without the blood and serum and the spirochetes, applied to the scarified skin of guinea pigs, were all negative. Where there was an actual multiplication or survival of the organism, as in the case of sterile suspensions or filtrates, with or without the addition of blood, the animal inoculations were positive.

Judging from the foregoing experiments, it seems highly improbable that, under natural conditions, the causative agent of icterohemorrhagic spirochetosis survives for any length of time after it has left the human body in the feces. It is probably rapidly destroyed by the common bacterial flora of the intestinal tract.

Polluted Water and Soil in Relation to Leptospira icterohæmorrhagiæ.

Samples of water were collected from the East River (a tidal river), from sewage, and from a stagnant cesspool in New York City. It is needless to say that such water is highly contaminated with various bacteria. In one series of experiments the water was used as it was, in another it was autoclaved in order to destroy contaminating bacteria, and in another it was filtered. An emulsion of freshly excreted horse stool was used in one series. The experimental data are given in Table III.

The results show that the spirochetes are not capable of multiplying or even of surviving for any length of time in these contaminated waters. They invariably disappeared in 48 hours. Even when the contaminating bacteria were removed by autoclaving or filtration and rabbit serum was added, only indifferent media resulted, and without the addition of an adequate amount of a suitable nutrient medium (rabbit serum in this experiment) no culture could be obtained.

The question of how long a rich culture of the spirochetes will remain viable when mixed with distilled water and left unprotected from dust in a room was next determined. A Flanders strain, having grown luxuriantly in rabbit serum, Ringer's solution, and agar mix-

TABLE III.

Leptospira icterohæmorrhagiæ introduced into.	Growth of bacteria.	Growth of spirochetes.	Survival of spirochetes.
Ringer's solution 4.5 cc. + rabbit serum 1.5 cc. (control)..	−	+++	Many wks.
East River water 6 cc.	+++	−	
“ “ “ 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
“ “ “ autoclaved, 6 cc.	−	−	
“ “ “ “ 4.5 cc. + rabbit serum 1.5 cc.	−	+	2 wks.
Sewer water 6 cc.	+++	−	
“ “ 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
“ “ autoclaved, 6 cc.	−	−	
“ “ “ 4.5 cc. + rabbit serum 1.5 cc..	−	+++	Many wks.
Stagnant water 6 cc.	+++	−	
“ “ 4.5 cc. + rabbit serum 1.5 cc	+++	−	
“ “ autoclaved, 6 cc.	−	−	
“ “ “ 4.5 cc. + rabbit serum 1.5 cc.	−	+++	Many wks.
Horse stool emulsion 6 cc.	+++	−	
“ “ “ 4.5 cc. + rabbit serum 1.5 cc.	+++	−	
“ “ “ autoclaved, 6 cc.	−	−	
“ “ “ “ 4.5 cc. + rabbit serum 1.5 cc.	−	+(?)	Accidentally contaminated.
Sewer filtrate 6 cc.	−	−	
“ “ 4.5 cc. + rabbit serum 1.5 cc.	−	++	More than 3 wks.

ture for 22 days, was placed in distilled water (ten times the volume of the culture) and then allowed to stand in the laboratory without being covered. The distilled water was not sterile, but contained a few large motile bacilli. The results were as follows:

24 hrs: Spirochetes +++; active and long; numerous motile bacilli; fluid slightly opalescent.
48 hrs.: Spirochetes +++; active; more bacilli.
3 days: “ ++; “ “ “
4 “ “ +; “ probably more bacilli.
5 “ “ +; many immobile; “ “
6 “ “ <+; nearly all dead.
7 “ “ −.

The spirochetes remained active and numerous for 48 hours, but all of them gradually disappeared within a week. A drinking water, therefore, richly contaminated with spirochetes, will not be infectious longer than a week.

Samples of soil were collected from several localities in and about New York City for use in an experiment performed to ascertain how long soil will harbor spirochetes under experimental conditions. The samples were rich in organic matter and some came directly from fertilized ground. They were all neutral in reaction. One specimen of soil was obtained from a deeper stratum than the others and was yellowish gray in color. All were purposely contaminated with the spirochetes and determinations of their continued presence in it made daily. No spirochetes could be detected after 72 hours, while there was always an abundance of bacteria. The spirochetes seem to be rapidly overgrown by the contaminating bacteria.

Various Bacteria in Relation to Leptospira icterohæmorrhagiæ.

When the spirochetes are excreted from the infected host, either in the feces or in the urine, their immediate fate will depend upon the presence of various putrefactive bacteria which are always found in the soil in which the feces or urine is deposited. Today we know all of the more common varieties of bacteria that inhabit the intestinal tract or that may be found in unclean objects or soil. There are, of course, a great number of anaerobes as well as aerobes, but since the spirochete in question is an obligatory aerobe,[4] the study of the relation of the bacteria to it becomes much simpler. We have, therefore, to direct our attention only to the part played by aerobic bacteria under natural conditions.

There are many ways of conducting such a study, but I have chosen an indirect one; namely, that of observing the effect of the simultaneous presence of the spirochete in question and each in turn of those bacteria which are likely to coexist with it at the moment when the infected feces, urine, or dead rodent becomes subject to the decomposing forces of the organic world.

A number of culture tubes containing media suitable for the growth

[4] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 593.

of spirochetes was prepared, and all were inoculated with the organism. The tubes were then inoculated with various bacteria and placed in a

TABLE IV.

Bacteria.	Growth of bacteria.	Growth of spirochetes.	Survival of spirochetes.	Remarks as to hemolysis in media.
Control without bacteria	−	+++	Many wks.	−
B. fæcalis alkaligenes	+	+++	12 days.	−
B. aerogenes	++	−	48 hrs.	+
B. cloacæ	++	−	24 "	<+
B. coli	++	−	24 "	<+
B. dysenteriæ Shiga	+	<+	48 "	−
B. " Flexner-Harris	<+	+	48 "	−
B. typhosus	<+	<+	48 "	−
B. paratyphosus A	++	−	24 "	−
B. " B	++	−	24 "	−
B. prodigiosus	++	−	24 "	+
B. proteus vulgaris	++	−	24 "	+
B. pyocyaneus	++	−	24 "	+
B. suipestifer	++	−	24 "	−
B. suicidus	++	−	24 "	−
B. subtilis	++	−	24 "	<+
B. mesentericus	+	−	24 "	<+
B. xerosis	+	−	24 "	−
B. sp.? large, motile, chromogenous	+	++	4 days.	−
Streptococcus Pr	<+	++	5 "	−
" Brown F 17	++	−	24 hrs.	−
" " A 1	±	++	5 days.	−
" " C 2	+	++	8 "	−
" " W 18	++	<+	3 "	−
" " K 4	++	++	5 "	+
" " S 6	++	<+	6 "	+
" " H 6	+	−	24 hrs.	+
Pneumococcus Type I	++	<+	3 days.	−
" " II	++	−	24 hrs.	−
" " III	+	<+	3 days.	−
" " IV	+	−	24 hrs.	−
Streptococcus aureus	++	+	48 "	<+ slowly.
" *albus*	++	+	48 "	−

thermostat at the temperature of 26°C. The culture media consisted of 1.5 cc. of rabbit serum, 4.5 cc. of Ringer's solution, 1 cc. of citrate plasma, and 1 drop of defibrinated rabbit blood. Observations were

made of the growth, survival, or disappearance of the spirochetes, the growth of the bacteria, and the presence or absence of hemolysis in the cultures. The results obtained during a period of 2 weeks are recorded in Table IV.

It is apparent from the recorded observations that the more vigorous the growth of a bacterium, the less is the possibility that the spirochetes in the same medium will multiply. The longest period of survival of the spirochetes, except in the control tubes, was observed in the media simultaneously inoculated with *Bacillus fœcalis alkaligenes.* Certain strains of streptococci, notably the non-hemolytic types, seem not to have interfered for a certain period, after which, however, the spirochetes rapidly disappeared from the culture. In the presence of most of the intestinal bacteria, such as *Bacillus coli*, *Bacillus aerogenes*, *Bacillus cloacæ*, etc., the spirochetes were not only unable to multiply but were rapidly destroyed within 24 hours. It may be added that no growth of the spirochetes took place in ordinary bouillon, either with or without the simultaneous inoculation of the bacteria just enumerated. The bacteria grew vigorously in the bouillon.

Microchemical Reactions.

The resistance of various spiral organisms to the solvent action of bile, bile salts, saponin, and sodium oleate has been a subject of study for many years, and it was once thought to differentiate the protozoa from the bacteria. Although this view is no longer valid, because some bacteria have been found to act like protozoa and *vice versa*, the fact is of sufficient interest to make worth while a determination of the resistance of the present organism to these reagents (Table V).

The jaundice spirochetes appear to be highly sensitive to the destructive action of the bile[5] and bile salts when employed in concentrations of 1:30 or more, while saponin exhibited no injurious effect upon them, even when used in as high a concentration as 10 per cent. The action of sodium oleate was stronger than that of the bile or bile salts and produced a granular disintegration of the organism in a dilution of 1:10,000. Among the organisms which under-

[5] Garnier, M., and Reilly, J., *Compt. rend. Soc. biol.*, 1917, lxxx, 41.

TABLE V.

Reagent.	Results in different concentrations.									
	1:10			1:30		1:100			1:300	1:1,000
	After 5 min.	After 30 min.	After 5 hrs.	After 5 min.	After 5 hrs.	After 5 min.	After 30 min.	After 5 hrs.	After 5 hrs.	After 5 hrs.
Ox bile.	Still active.	None motile, nearly all shadow forms.			All shadow forms.			Some affected. Nearly all active.	No effect. All active.	
Rabbit bile.	Many inactive.	All shadow forms.			All shadow forms.			All shadow forms.	Some affected. Majority active.	
Sodium taurocholate.	All shadow forms.	Shadow forms.	Shadow forms less distinct.	All immobile. Better preserved in form.		Nearly all active.	All shadow forms.		Nearly all active.	No effect. All active.
Sodium glycocholate.	Nearly all shadow forms.		All shadow forms.		All shadow forms.			Some still active.	Nearly all active.	No effect. All active.
Sodium oleate.	All dead; distorted and granular.			All dead; distorted and granular.				Nearly all gone; few motile.	Nearly all gone; few motile.	Nearly all gone; few motile, but more active than those in the 1:300 dilution.
Saponin.			No effect. All active.		No effect. All active.			No effect. All active.	No effect. All active.	

went this disintegration, however, was a number of actively motile, apparently intact organisms.

The destructive action of the rabbit bile as well as of the bile salts and sodium oleate was considerably reduced by the addition of serum, as shown in Table VI.

Ido and his coworkers[1] observed that in spite of the difficulty of finding spirochetes in the bile when it was examined under the dark-field microscope, two out of three specimens of the bile of guinea pigs dying of experimental spirochætosis icterohæmorrhagica were capable of producing typical infection in the guinea pig. This

TABLE VI.

Leptospira icterohæmorrhagiæ introduced into.	10 per cent rabbit bile 1 cc. + culture 1 cc.	10 per cent sodium taurocholate 1 cc. + culture 1 cc.	10 per cent sodium oleate 1 cc. + culture 1 cc.	0 + culture 1 cc.
Rabbit serum 0.5 cc.	No apparent effect. All active.	Nearly all active.	Nearly all active.	All motile.
60 per cent rabbit serum 0.5 cc.	Many gone, some motile.	Many dead and distorted. A few motile.	Many active.	" "
20 per cent rabbit serum 0.5 cc.	Nearly all gone.	Nearly all gone.	" "	" "
6 per cent rabbit serum 0.5 cc.	All gone.	All gone.	Nearly all gone.	" "
2 per cent rabbit serum 0.5 cc.	" "	" "	All gone.	" "
Ringer's solution 0.5 cc.	" "	" "	" "	" "

may be ascribed to the fact that in these specimens of bile there was mixed a certain amount of the blood and also the serous exudate from the affected liver, which, by virtue of their well known inhibitory effect upon the solvent action of the bile salts, must have protected some spirochetes from destruction in the bile. Guinea pig bile was affected by the serum in the same way.

A parallel series of experiments with a specimen of ox bile obtained from an abattoir gave somewhat contradictory results. In this instance the addition of the rabbit or horse serum failed to check the destruction of the organism by this bile, which had a much stronger

solvent power than that of the rabbit or guinea pig. At all events, the amount of the serum necessary to nullify the destructive action of the bile is so large that the escape of the spirochetes in the bile seems less probable than would appear from the observations of the investigators just quoted. Perhaps the impairment of hepatic function through the spirochetal infection of the organ may lead to a decrease of the bile salts in such a specimen.

Leptospira icterohæmorrhagiæ and Intermediary Hosts.

It has been shown by previous investigators that the spirochetes may remain in the organs of certain rodents without producing serious illness, and that they may be excreted in the urine. From the experiments already described, it seems improbable that the spirochete can survive very long after leaving the infected hosts. The infection of man, therefore, must result from contact with the spirochete before its destruction under natural conditions; that is, the carrier rodents must be present in places frequented by man. But while this source of infection may explain many cases of infection, there are a few in which the infective agent cannot be traced in this way.

The question of insect carriers has been taken up by Reiter,[6] who obtained only negative results with certain biting flies, fleas, and bedbugs. In the present study opportunities were afforded the writer to ascertain whether or not the larvæ of certain varieties of flies or mosquitoes could become infected with spirochetes when fed on infected guinea pig liver or raised in a stagnant water tank into which an abundance of the culture had been put.

The larvæ of the common house-fly were allowed to feed for 2 days on infected material consisting of several pieces of the liver and kidney of a guinea pig killed in the last stage of experimental Weil's disease. They were then transferred to a clean receptacle and fed for 5 days on a non-infected mass of horse manure, and at the end of that time they were crushed into an emulsion and smeared over depilated areas of the skin of guinea pigs. The emulsion was also examined for spirochetes under the dark-field microscope. The examination revealed no spirochetes, and the guinea pig remained normal.

[6] Reiter, H., *Deutsch. med. Woch.*, 1916, xlii, 1282.

A similar experiment with the larvæ of bluebottle flies (*Calliphora vomitoria*) gave only negative results.

In another series of experiments, about 50 cc. of a rich culture of spirochetes (Japanese strain) were added to 150 cc. of stagnant water in which twenty-five mosquito larvæ had been living for some time. The water was neutral in reaction and was quite clear and transparent at the time when the culture was introduced. The larvæ swam about actively in the usual manner after the addition of the culture. A drop of the contaminated water examined under the dark-field microscope contained numerous active spirochetes. There were a few bacteria. After 24 hours at room temperature, the water became somewhat turbid. Most of the larvæ were still active, but the number of the spirochetes was diminished and that of the bacteria increased. At the end of 48 hours there was a scum of bacteria over the surface of the water and no spirochetes could be found. All but six of the largest larvæ had died. The water was full of bacteria and infusoria. It is possible that the death of the mosquito larvæ and of the spirochetes was the result of overcrowding by the bacteria and infusoria, increased suddenly by the addition of the culture media to the water. The surviving larvæ were kept in the same water for 5 days and then crushed into an emulsion to be used for an infection experiment on a guinea pig and also for examination under the dark-field microscope. The results were entirely negative.

Another series of experiments was performed with adult mosquitoes (*Culex pipiens*) by first allowing them to feed on an infected guinea pig, in the blood of which had been found spirochetes, and then, after 6 days, causing them to bite normal guinea pigs. No infection resulted from their bites.

Wood ticks (*Dermacentor andersoni*) failed in several experiments to transmit the infection from guinea pig to guinea pig. Leeches (*Hirudo medicinalis*) were allowed to suck blood from an infected guinea pig until their bodies were engorged. In the blood escaping from the wound inflicted by the leeches a few spirochetes could be found under the dark-field microscope. These "infected" leeches were kept at room temperature for 7 days and afterwards in a cool room at 15°C., being taken out at the end of intervals of 2, 3, 4, 6, and 8 months and made to suck normal guinea pigs, but so far no infection

has been produced. Some of the leeches died in the meantime, but those which still survived at the end of the 3 month interval were examined for the presence of spirochetes. The viscid, dark reddish, decomposed (?) blood showed no spirochetes under the dark-field microscope, nor did it cause infection when tested on guinea pigs. Some of the tissues were examined by the silver impregnation method, but with negative results. Apparently there is no multiplication of the spirochetes after their ingestion by leeches, and no infection can be induced by the bite of the latter after a period of 1 week.

SUMMARY AND CONCLUSIONS.

1. *Leptospira icterohæmorrhagiæ* is unable to grow in the urine, either with or without the addition of suitable culture ingredients, the acidity of the urine being detrimental to the growth. It survives less than 24 hours, unless the urine is neutralized or slightly alkalized, when the period of survival is somewhat longer. If suitable nutrient ingredients are added to the neutralized or slightly alkalized urine, the organism is able to grow for about 10 days, after which multiplication ceases.

2. Feces from normal or jaundiced persons destroy *Leptospira icterohæmorrhagiæ* within 24 hours when a rich culture is added and the mixture allowed to stand at 26°C. The addition of blood serum and corpuscles does not prevent the destruction of the organism. Autoclaved specimens and filtrates of unheated feces do not constitute a suitable medium in which to keep the organism alive for any length of time, but the addition of blood corpuscles and serum in adequate quantities renders them fairly satisfactory as media. Under natural conditions *Leptospira icterohæmorrhagiæ* cast off in the feces cannot survive more than 24 hours.

3. Polluted water, sewage, and soil will not serve to keep *Leptospira icterohæmorrhagiæ* alive for more than 3 days at the most. When deprived by filtration or autoclaving of their bacteria they become indifferent diluents and may be used to make up a culture medium when mixed with serum and citrate plasma of a suitable animal. Sterilized soil with a neutral reaction, when added to a culture, has an unfavorable effect upon the growth of the organism.

4. Most of the aerobic bacteria found in feces, sewage, soil, and tap water inhibit the growth of *Leptospira icterohæmorrhagiæ* when inoculated into the same medium. *Bacillus fæcalis alkaligenes* and many strains of non-hemolytic streptococci caused the least interference, although growth was never so vigorous or lasting in the media in which they were present as in the control media. Certain pathogenic bacteria (*Bacillus typhosus*, *Bacillus paratyphosus*, *Bacillus dysenteriæ*, pneumococcus) are antagonistic to the growth of the spirochete.

5. *Leptospira icterohæmorrhagiæ* is highly sensitive to the destructive action of bile, bile salts, and sodium oleate, but resists the action of saponin. In this last respect it differs from many so called spirochetes. The destructive action of these agents is counteracted by blood serum.

6. The larvæ and adults of the *Culex* mosquito, the larvæ of the house-fly and bluebottle fly, wood ticks (*Dermacentor andersoni*), and leeches failed to become carriers of the spirochetes when fed on infected guinea pigs or their organs; that is, they cannot play the part of an intermediary host of *Leptospira icterohæmorrhagiæ*.

BEHAVIOR OF HYPOCHLORITE AND OF CHLORAMINE-T SOLUTIONS IN CONTACT WITH NECROTIC AND NORMAL TISSUES IN VIVO.

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It has been known for some time that the chlorine content and consequently the potency of hypochlorite of soda solutions diminishes rapidly when in contact with the surface of wounds. This is emphasized by Carrel and Dehelly, and for this reason they advocate a frequent renewal of the antiseptic solution to the wound.[1] This insures that the concentration shall be kept as constant as possible.

It would be difficult to determine the rapidity of the fall in chlorine concentration on an actual wound as encountered in the ward, and almost impossible to parallel such observations with others on an equal quantity of solution in contact with an equal area of normal skin. Inasmuch as exact determinations of the rapidity of the fall in chlorine concentration on pathological and on normal skin, under experimental conditions, might be of value to surgeons using Dakin's hypochlorite and chloramine-T solutions clinically, we chose the following method of investigation.

The left ears of three white rabbits of the same relative size and weight were exposed to the rays emitted by a Coolidge tube. The spark-gap used measured 3 inches; the milliamperage was 10; the distance from the target to the ear was 6 inches; and the time of exposure was 20 minutes.

8 weeks later the x-rayed ears each exhibited a sharply demarcated gangrenous area over which there were considerable crusting of epithelium and secretions and in the lumen there was much thick pus.

The ears of the affected rabbits were each suspended for 20 minutes in a beaker containing 400 cc. of the solution to be tested.

[1] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 65.

Rabbit 1.—Right and left ears suspended in separate beakers containing Dakin's hypochlorite solution, made from bleaching powder, 10 cc. of which required 13 cc. of 0.1 N sodium thiosulfate solution for reduction (0.48 per cent sodium hypochlorite).

Rabbit 2.—Right and left ears suspended separately in beakers containing a solution comparable in alkalinity with properly made Dakin's hypochlorite solution—sodium carbonate 1 gm. and sodium bicarbonate 17 gm. per liter of water.

Rabbit 3.—Right and left ears suspended separately in chloramine-T[2] solution which required 12.75 cc. of sodium thiosulfate for reduction (about 2 per cent chloramine-T), and contained approximately the same proportion of available chlorine as the hypochlorite solution used on Rabbit 1.

TABLE I.

Solution.	In contact with.	Before.	Immediately after.	2 hrs. after.	17 hrs. after.
		cc.	*cc.*	*cc.*	*cc.*
Dakin's hypochlorite solution........	Normal ear.	13.00	12.35	12.15	11.50
" " "	Gangrenous ear.	13.00	11.55	10.30	8.65
" " "	Control (no tissue).	13.00	13.00	13.05	12.60
Carbonate-bicarbonate "	Normal ear.				
" "	Gangrenous ear.				
Chloramine-T solution..............	Normal ear.	12.75	12.75		12.75
" "	Gangrenous ear.	12.75	12.75		12.35
" "	Control (no tissue).	12.75	12.75		12.75

In the tables the figures represent the number of cubic centimeters of 0.1 N sodium thiosulfate solution required to reduce the chlorine in 10 cc. of the solution.

Table I shows the titration figures before, immediately after the 20 minutes' exposure of the normal and necrotic ears to the solutions, 2 hours after the ears had been removed from the solutions, and 17 hours after removal. The solutions were kept in covered vessels at room temperature in the interval between titrations.

The fall in chlorine concentration was more rapid in the Dakin's hypochlorite solution applied to the gangrenous ear than in that applied to the normal ear. The fall in concentration, however, was not complete immediately after the ears were removed from the solution but became more pronounced the longer the interval between

[2] Prepared by the Abbott Laboratories, Chicago.

the removal of the ears and the titration. The titration of the control solution, which had not been exposed to any tissue, demonstrated a fall in the titration figure from 13 to 12.60 cc., and a small proportion of the loss in chlorine of the solutions in contact with the tissues might be explained by this spontaneous deterioration of the unstable hypochlorite solutions. However, this factor is insufficient to account for the fall from 13 cc. before exposure to 8.65 cc. 17 hours later in the gangrenous ear, nor from the same figure before to 11.50 cc. at the end of the 17 hour interval in the normal ear. The fall from 13 cc. before exposure to 11.55 cc. immediately afterward is associated with the erosive action of the hypochlorite solutions, which we have measured quantitatively in a former investigation,[3] but the cause of the further fall to 8.65 cc. is not immediately clear. The fluid in contact with the gangrenous ear was cloudy immediately after the removal of the ear. This cloudiness of the fluid was not so marked at the end of the 2 hour interval when the second titration was made, and the fluid was almost as clear as the control at the end of the 17 hour interval when the last titration gave the lowest chlorine concentration recorded. Close inspection of the fluid immediately after the removal of the ear revealed the presence of small particles of necrotic tissue, flecks of pus, etc., in suspension. These became less noticeable the longer the antiseptic solution was allowed to act. The fall in chlorine concentration exhibited immediately after the removal of the ears was due to the erosive effect of the solution on the necrotic tissue, and to its combination with the products of the tissues *in situ*. However, during this action, appreciable particles of necrotic tissue, agglomerations of pus cells, and little gummy concretions made up of dried serum, epithelial cells, etc., were separated from the necrotic ear and it is the subsequent reaction of the hypochlorite with these which caused the continued fall in the chlorine titer. That the chlorine is directly concerned in this solvent action seems assured from former experiments reported.[3] In the course of the reaction the chlorine probably goes into such stable union with the protein substances that it is not available to the sodium thiosul-

[3] Taylor, H. D., and Austin, J. H., The solvent action of antiseptics on necrotic tissue, *J. Exp. Med.*, 1918, xxvii, 155.

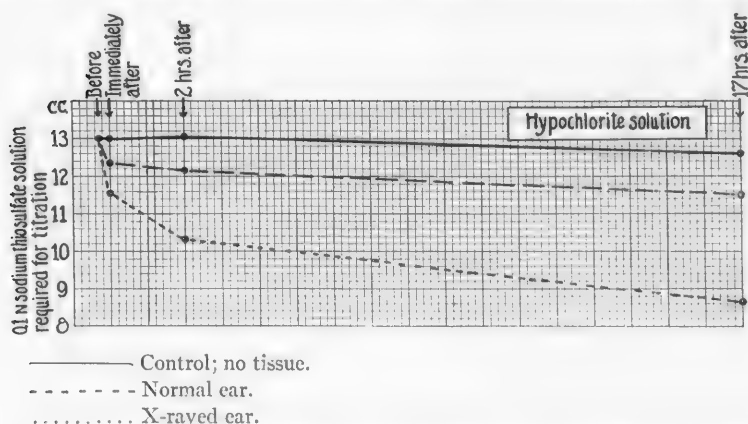
fate solution, and thus the fall in titer measures the exact quantity of chlorine used up in the reaction with the wound products.

The fall in chlorine concentration of the hypochlorite solution in contact with the normal ear, much less pronounced than with the x-rayed ear, was probably due to the erosive action of the solution on the hair and superficial epithelium of the normal ear and the slow digestion of the particles removed caused the slow fall noted over the interval of 17 hours. As there was much less tissue capable of reacting with the solution in the normal than in the gangrenous ear, the titration figures were higher at all observations in the solution exposed to the former. Close observation revealed the erosive effect on the ear itself.

The fall in chlorine concentration noted in the chloramine-T solutions was much less than that observed in the hypochlorite solutions. This corresponds with the greater stability of the former and with their lack of erosive effect on necrotic tissue.[3] It is interesting to note that there was no fall in chlorine concentration in the chloramine-T solution applied to the normal ear, and correspondingly no erosive action on the hair or superficial epithelium was demonstrable. The solution was likewise clear when removed from the ear and throughout the period of observation, in contrast to the hypochlorite solution which was at first cloudy and only late in the experiment became relatively clear. The titration figures after 17 hours were approximately the same as those made immediately after the removal of the ears from the solution. Text-fig. 1 shows graphically the fall in chlorine concentration in the hypochlorite solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue. Text-fig. 2 gives comparable curves for the chloramine-T solutions.

A weaker hypochlorite solution, titrating 9 cc. of sodium thiosulfate (0.1 N), was applied to the gangrenous ear and to the normal ear of Rabbit 1, and titration figures before, immediately after removal of the ears from the solution, 2 hours afterward, and 17 hours afterward were compared with a control solution which was never in contact with tissue. These results, shown in Table II and Text-fig. 3, confirm those shown in Table I and Text-fig. 1 for Rabbit 1.

The ears of each rabbit were then suspended in solutions of the same types and concentrations as those shown in Table I for 7 consecutive days, the period of exposure on each day being 20 minutes. At the end of this time it was seen that the gangrenous ear suspended in Dakin's hypochlorite solution had cleared up proportionately more



———— Control; no tissue.
- - - - - - - Normal ear.
.... X-rayed ear.

TEXT-FIG. 1. The fall in chlorine concentration in the hypochlorite solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue.

TABLE II.

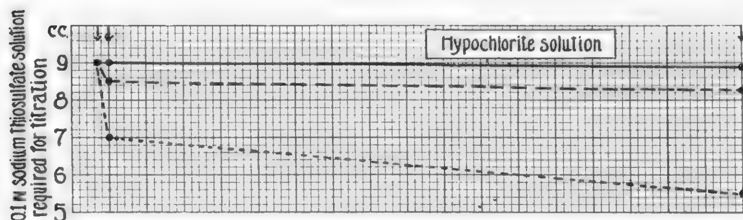
Solution.	In contact with.	Before.	Immediately after.	17 hrs. after.
		cc.	*cc.*	*cc.*
Dakin's hypochlorite solution..........	Normal ear.	9.0	8.5	8.25
" " "	Gangrenous ear.	9.0	7.0	5.5
" " "	Control (no tissue).	9.0	9.0	8.9

than the gangrenous ears of either of the other rabbits. The scabs were more eroded and the pus present was less in amount than in either of the others. The gangrenous area looked much cleaner. The chloramine-T gangrenous ear, which was not so severe an x-ray burn in the beginning, had not improved appreciably. The gangrenous ear

treated with the control alkaline solution had not changed in appearance.

The normal ears of the chloramine-T and of the alkaline control rabbits were just as they had been before treatment with these substances. The normal ear treated with the hypochlorite solution was

TEXT-FIG. 2.



TEXT-FIG. 3.

TEXT-FIG. 2. The fall in chlorine concentration in the chloramine-T solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue.

TEXT-FIG. 3. The fall in chlorine concentration of the second hypochlorite solution, titrating 9 cc. of sodium thiosulfate (0.1 N), applied to the gangrenous ear, in that applied to the normal ear, and in the control which was not allowed to act on any tissue.

intensely inflamed. It was twice as thick as it had been before it was treated, due to intense edema. Congestion was marked and the surface temperature was higher than normal. There was superficial ulceration in places and petechiæ were scattered through the subcutaneous tissues.

CONCLUSIONS.

1. The fall in chlorine concentration of Dakin's hypochlorite solution is more rapid in contact with necrotic than in contact with normal tissue.

2. The fall in chlorine concentration of chloramine-T solution is very slight when applied to necrotic tissue and is negligible when applied to normal tissue.

3. The action of the hypochlorite solution on tissue results in the separation of particles of necrotic tissue, hair, epithelial scales, coagulated serum, etc., and a gradual digestion of these substances, taking place over a period of at least 17 hours.

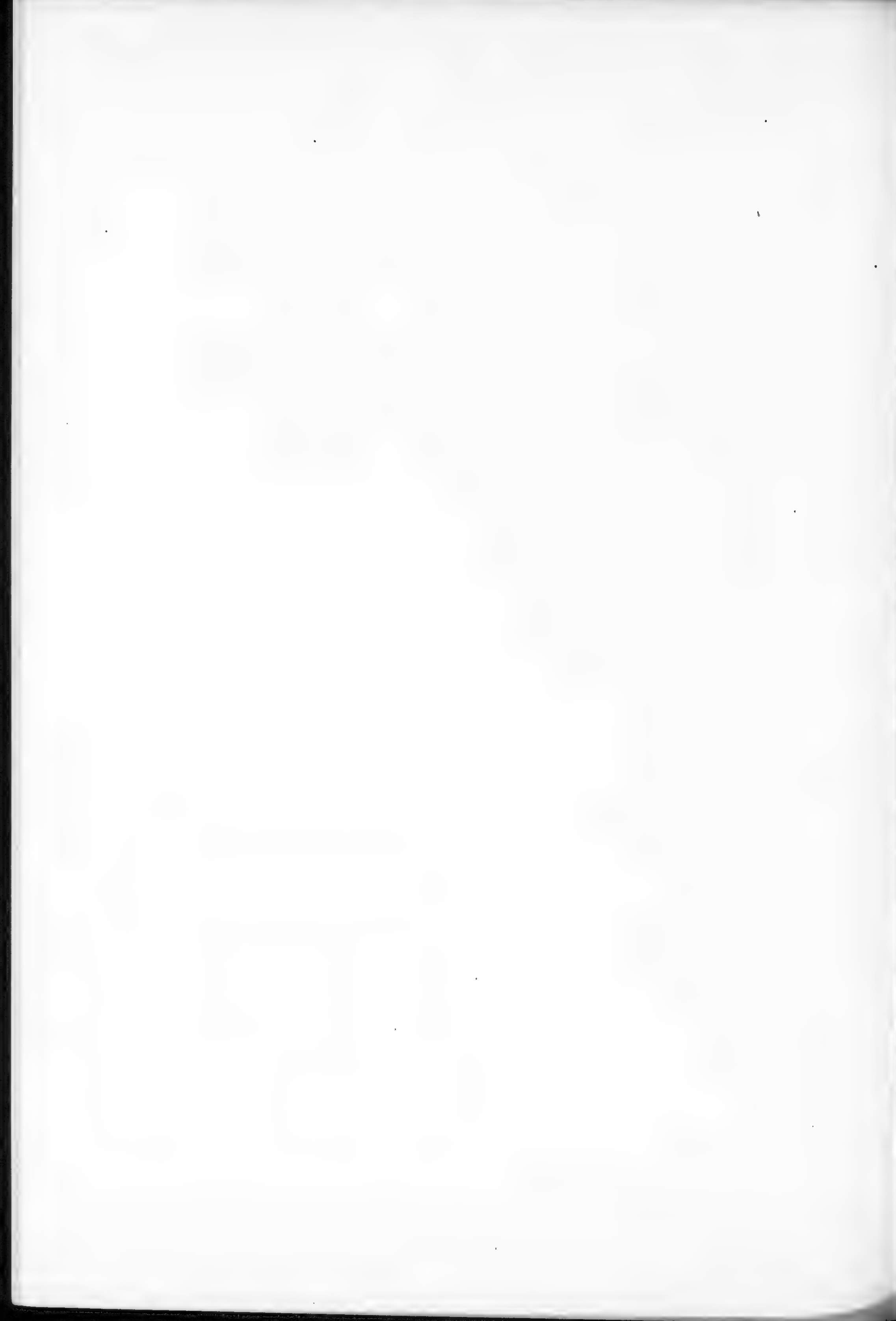
4. The fall in the chlorine concentration of the hypochlorite solution is not complete until the particles are completely dissolved.

5. Chloramine-T solution, 2 per cent, has no erosive effect comparable with that exhibited by the hypochlorite solution.

6. Repeated exposures to the three solutions show the hypochlorite solution to be superior in its cleansing ability on necrotic tissue.

7. The hypochlorite solution is much more irritating to normal rabbit skin than chloramine-T solution or the alkaline control solution.

8. Therefore, the irritating effects must be due to the readily available chlorine.



TOXICITY OF CERTAIN WIDELY USED ANTISEPTICS.

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In view of the widespread use of certain antiseptics in the treatment of infected wounds, it has seemed desirable to make toxicity tests on animals under conditions in which rapid absorption might be expected. While, as a rule, the antiseptics are employed under conditions that preclude the possibility of much absorption with consequent systemic effect, yet occasionally certain of them have been recommended and in some instances even used for injection into closed cavities. This practice, which would probably lead to considerable absorption, has not been general, the majority of surgeons proceeding with much caution.

A few experiments of Carrel and Dehelly[1] demonstrated that Dakin's hypochlorite solution when injected subcutaneously in the guinea pig was relatively non-toxic, one-fortieth of the body weight of the animal injected being borne without demonstrable ill effect. Bashford[2] has tested the toxic effect of dilute hypochlorite solutions on the living tadpole immersed in Dakin's solution. Inasmuch as these experiments were few and no data for comparing the relative toxicity of a series of antiseptics were given, it was decided to investigate the toxic action of a number of antiseptic substances in common use. The method was to inject increasing doses into mice intraperitoneally and into guinea pigs both subcutaneously and intraperitoneally, and to note and tabulate the results.

Method.

White mice of approximately 20 gm. and guinea pigs of 300 to 600 gm. body weight were employed and the amount of chemical used is

[1] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 32.

[2] Bashford, E. F., *Lancet*, 1917, ii, 595.

based on the weight of each animal. In regard to the chlorinated antiseptics, the lethal dose is calculated in terms of the sodium hypochlorite equivalent and the available chlorine of the antiseptic. The percentage of the total weight of the antiseptic substance represented by the sodium hypochlorite equivalent is given in a foot-note to Table I.

In Table I the results of the intraperitoneal injection in mice of increasing doses of the antiseptics studied are tabulated. In Column A of Table IV will be found a condensed summary of these results, the antiseptics being arranged in the order of their decreasing toxicity for the animals. Control injections of four of the vehicles employed, namely water, isotonic saline solution, sterile paraffin oil, and Dakin and Dunham's bland oil solvent for dichloramine-T (chlorcosane[3]), show these to be well borne in larger doses than those employed in any of the injections with antiseptics into the test animals. Eucalyptol, however, which has been used in combination with paraffin oil as a vehicle for dichloramine-T is so toxic that its use in experiments of the nature of those recorded here is impossible. The diluting vehicle has been water or isotonic saline solution with all antiseptics except dichloramine-T.

In Table I are given in detail the results of the experiments in which mice were used. The nature and strength of the solution injected, the amount of the solution in cubic centimeters, the amount of the drug in actual milligrams administered and in milligrams per 100 gm. of body weight, with the final results, are recorded.

Table II gives in the same way the results obtained with the few guinea pigs that were injected subcutaneously.

In Table III are recorded the results of the experiments in which guinea pigs were injected intraperitoneally. The form of the table is the same as in Tables I and II.

Table IV summarizes the results given in Tables I to III, showing the greatest dose per 100 gm. of body weight that the animals were able to survive and the smallest dose necessary to kill with the antiseptics named.

[3] The chlorcosane was kindly given us by Dr. H. D. Dakin and Dr. E. K. Dunham. Dakin, H. D., and Dunham, E. K., *Brit. Med. J.*, 1918, i, 51.

DISCUSSION.

It will be seen from the tables that the only antiseptic of which the smallest fatal dose was smaller than the largest survival dose was dichloramine-T. Since two mice survived 4.7 mg. per 100 gm. of body weight, it is probable that 15.5 mg. rather than 1.6 mg. is to be considered the smallest fatal dose for this series. The distribution of the drug in the viscid bland oil used as a vehicle is probably uneven, which may account for the somewhat variable results obtained with this antiseptic both in mice and in guinea pigs.

Of all the substances tested, eucalyptol and brilliant green are the most toxic, the lethal dose of each being 0.1 mg. per 100 gm. of body weight. Mercurophen,[4] mercuric chloride, and chloramine-T constitute the group with the next highest toxicity, the lethal dose being 1 mg. per 100 gm. of body weight. Dichloramine-T, proflavine,[5] and the four hypochlorite solutions tested follow in the order named with a lethal dose of about 10 to 15 mg. per 100 gm. of body weight. The least toxic chemicals are iodine and phenol, of which the lethal doses are about 50 mg. per 100 gm. of body weight.

In Table II are recorded a few experiments with the antiseptics injected under the skin of the abdomen of guinea pigs. The lethal dose of Dakin's hypochlorite solution per 100 gm. of body weight is the same as that determined intraperitoneally in the mouse. Chloramine-T and dichloramine-T administered in this manner gave rise to local necrosis with extensive sloughing. It is probable that only a small part of the drug injected reached the general system of the animal and in consequence the determination of the lethal dose in this way can hardly be considered satisfactory. It was accordingly abandoned and five of the antiseptics were tested in guinea pigs by intraperitoneal injections. The results are tabulated in Table III and summarized in Column B of Table IV.

Chloramine-T has the same toxicity per unit of body weight for guinea pigs and for mice. The same may be true of dichloramine-T or this substance may be somewhat less toxic for the guinea pig.

[4] The mercurophen was sent to us for trial through the kindness of Dr. J. F. Schamberg.

[5] The proflavine was obtained from England.

TABLE I.

Results of Injecting Mice Intraperitoneally.

Animal No.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
		cc.		*mg.*	*mg.*	
1	Sodium chloride 0.85 per cent	0.50	Sodium chloride.	4.20	20.00	Lived.
2	" " 0.85 " "	1.00	" "	8.50	40.00	"
3	" " 0.85 " "	4.00	" "	34.00	170.00	"
4	Distilled water	0.50				Lived.
5	" "	2.00				"
6	" "	4.00				"
7	Dakin's hypochlorite 0.41 per cent (bleaching powder)	0.10	Sodium hypochlorite.	0.40	2.00	Lived.
8	" " 0.41 " " " "	0.50	" "	2.00	10.00	"
9	" " 0.47 " " (original)	0.50	" "	2.30	12.00	"
10	" " 0.49 " " (bleaching powder)	0.50	" "	2.40	12.00	"
11	" " 0.50 " " (neutral)*	0.50	" "	2.50	12.00	"
12	" " 0.47 " " (original)	1.00	" "	4.70	24.00	Died in 15 hrs.†
13	" " 0.50 " " (neutral)*	1.00	" "	5.00	25.00	" " 18 " †
14	" " 0.41 " " (bleaching powder)	2.00	" "	8.20	40.00	" " 3 "
15	" " 0.47 " " (original)	2.00	" "	9.40	47.00	" " 15 " †
16	" " 0.41 " " (bleaching powder)	4.00	" "	16.40	80.00	" " 1½ "
17	Hychlorite, 0.5 per cent sodium hypochlorite	0.33	Sodium hypochlorite.	1.70	8.00	Lived.
18	" 0.5 " " " "	0.33	" "	1.70	8.00	"
19	" 0.5 " " " "	0.50	" "	2.50	12.00	"
20	" 0.5 " " " "	0.50	" "	2.50	12.00	Died in 6 hrs.

21	Chloramine-T 0.2 per cent	0.10	Sodium hypochlorite.	0.05	0.26	Lived.
22	" 0.2 " "	0.30	" "	0.16	0.80	"
23	" 2.0 " "	0.10	" "	0.50	2.60	Died in 19½ hrs.
24	" 2.0 " "	0.50	" "	2.60	13.00	" " 2 "
25	" 2.0 " "	0.50	" "	2.60	13.00	" " 1½ "
26	" 2.0 " "	0.50	" "	2.60	13.00	" " 2½ "
27	Dichloramine-T 0.5 per cent in bland oil	0.10	Sodium hypochlorite.	0.30	1.60	Lived.
28	" 0.5 " " " " "	0.10	" "	0.30	1.60	Died in 30 hrs.
29	" 0.5 " " " " "	0.30	" "	0.90	4.70	Lived.
30	" 0.5 " " " " "	0.30	" "	0.90	4.70	"
31	" 5.0 " " " " "	0.10	" "	3.10	15.50	Died in 18 hrs.†
32	" 5.0 " " " " "	0.10	" "	3.10	15.50	" " 18 "†
33	" 5.0 " " " " "	0.20	" "	6.20	31.00	" " 18 "†
34	" 5.0 " " " " "	0.20	" "	6.20	31.00	" " 18 "†
35	" 5.0 " " " " "	0.33	" "	10.60	50.00	" " 2 "
36	" 5.0 " " " " "	0.50	" "	15.50	75.00	" " 1½ "
37	" 5.0 " " " " "	0.50	" "	15.50	75.00	" " 1½ "
38	Paraffin oil	0.50				Lived.
39	Bland oil	0.33				"
40	" "	0.50				"
41	Mercuric chloride 0.01 per cent	0.10	Mercuric chloride.	0.01	0.05	Lived.
42	" " 0.01 " "	0.30	" "	0.03	0.15	"
43	" " 0.10 " "	0.10	" "	0.10	0.50	"
44	" " 0.10 " "	0.50	" "	0.50	2.50	Died in 18 hrs.
45	Mercurophen 0.01 per cent	0.50	Mercurophen.	0.05	0.25	Lived.
46	" 0.10 " "	0.10	"	0.10	0.50	"
47	" 0.10 " "	0.30	"	0.30	1.50	Died in 48 hrs.
48	" 0.10 " "	1.00	"	1.00	5.00	" " 27 "

* Cullen, G. E., and Austin, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 41.

† Died over night.

TABLE I—*Continued.*

Animal No.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
		cc.		mg.	mg.	
49	Brilliant green 0.01 per cent.	0.10	Brilliant green.	0.01	0.05	Lived.
50	" " 0.01 " "	0.30	" "	0.03	0.15	Died in 48 hrs.
51	" " 0.10 " "	0.10	" "	0.10	0.50	" " 6 "
52	" " 0.10 " "	0.50	" "	0.50	2.50	" " 18 "
53	" " 0.10 " "	0.50	" "	0.50	2.50	" " 3½ "
54	Proflavine 0.1 per cent.	0.33	Proflavine.	0.33	1.70	Lived.
55	" 1.0 " "	0.10	"	1.00	5.00	"
56	" 1.0 " "	0.50	"	5.00	25.00	Died in 18 hrs.†
57	" 1.0 " "	0.50	"	5.00	25.00	" " 12 "
58	" 1.0 " "	1.00	"	10.00	50.00	" " 3 "
59	" 1.0 " "	1.00	"	10.00	50.00	" " 18 "
60	Tincture of iodine 7.0 per cent.	0.10	Iodine.	7.00	35.00	Lived.
61	" " " 7.0 " "	0.30	"	21.00	100.00	Died in 45 min.
62	" " " 7.0 " "	0.50	"	35.00	175.00	" " 10 "
63	" " " 7.0 " "	1.00	"	70.00	350.00	" " 15 "
64	Commercial hypochlorite (Javelle water) 0.5 per cent of sodium hypochlorite.	0.50	Sodium hypochlorite.	2.50	12.00	Lived.
65	Commercial hypochlorite (Javelle water) 1.0 per cent of sodium hypochlorite.	0.50	" "	5.00	25.00	Died in 4½ hrs.
66	Commercial hypochlorite (Javelle water) 1.0 per cent of sodium hypochlorite.	1.00	" "	10.00	50.00	" " 2½ "

67	Peroxide of hydrogen 3.0 per cent (commercial)	0.50	Peroxide of hydrogen.	15.00	75.00	Lived.
68	Magnesium hypochlorite equivalent to 0.5 per cent sodium hypochlorite	0.50	Sodium hypochlorite.	2.50	12.00	Lived.
69	Magnesium hypochlorite equivalent to 1.0 per cent sodium hypochlorite	0.50	" "	5.00	25.00	Died in 25 min.
70	Magnesium hypochlorite equivalent to 1.0 per cent sodium hypochlorite	1.00	" "	10.00	50.00	" " 10 "
71	Phenol 0.25 per cent	0.50	Phenol.	1.25	6.20	Lived.
72	" 1.00 " "	0.30	"	3.00	15.00	"
73	" 1.00 " "	0.50	"	5.00	25.00	"
74	" 1.00 " "	1.00	"	10.00	50.00	"
75	" 1.00 " "	1.00	"	10.00	50.00	Died in 5 min.
76	" 1.00 " "	1.50	"	15.00	75.00	" " 12 "
77	Eucalyptol 10.0 per cent in paraffin oil	0.10	Eucalyptol.	0.01	0.05	Lived.
78	" 10.0 " " " " "	0.30	"	0.03	0.15	Died in 4 hrs.
79	" 100.0 " "	0.10	"	0.10	0.50	" " 10 min.
80	" 50.0 " " in paraffin oil	0.50	"	0.25	1.25	" " 10 "

Sodium hypochlorite equivalent of

Dakin's hypochlorite	= 100	per cent.
Commercial hypochlorite (Javelle water)	= 100	" "
Hychlorite	= 100	" "
Magnesium hypochlorite	= 117.4	" "
Chloramine-T	= 26.5	" "
Dichloramine-T	= 62.1	" "

TABLE II.

Results of Injecting Guinea Pigs Subcutaneously.

Animal No.	Weight.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
	gm.		*cc.*		*mg.*	*mg.*	
1	400	Dakin's hypochlorite 0.5 per cent	10.0	Sodium hypochlorite.	50.00	12.00	Lived.
2	550	" " 0.5 " "	13.7	" "	68.00	12.00	"
3	425	" " 0.48 " "	10.6	" "	51.00	12.00	"
4	475	" " 0.5 " "	11.9	" "	60.00	13.00	"
5	450	" " 0.48 " "	22.5	" "	108.00	24.00	Died in 12 hrs.
6	350	Hychlorite, 0.5 per cent sodium hypochlorite	9.0	Sodium hypochlorite.	45.00	13.00	Lived.
7	450	Chloramine-T 2.0 per cent	11.25	Sodium hypochlorite.	59.00	13.00	Lived (sloughed).
8	550	" 2.0 " "	13.7	" "	73.00	13.00	" "
9	450	Eucalyptol 50.0 per cent	11.25	Eucalyptol.	5,600.00	1,200.00	Died in 12 hrs.
10	350	Dichloramine-T 5.0 per cent in bland oil	9.0	Sodium hypochlorite.	280.00	83.00	Lived (sloughed).
11	575	Proflavine 0.1 per cent	7.2	Proflavine.	7.20	1.25	Lived.
12	300	" 0.1 " "	7.5	"	7.50	2.50	"

TABLE III.

Results of Injecting Guinea Pigs Intraperitoneally.

Animal No.	Weight.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
	gm.		*cc.*		*mg.*	*mg.*	
13	507	Dakin's hypochlorite 0.5 per cent	3.10	Sodium hypochlorite.	15.00	3.00	Lived.
14	350	" " 0.5 " "	4.37	" "	22.00	6.30	Died in 9½ hrs.
15	375	" " 0.5 " "	9.37	" "	47.00	12.50	" " 7½ "
16	566	Hychlorite, 0.5 per cent sodium hypochlorite.	3.50	Sodium hypochlorite.	17.00	3.00	Lived.
17	500	" 0.5 " " " "	6.25	" "	31.00	6.20	Died in 3¼ hrs.
18	450	" 0.5 " " " "	11.25	" "	56.00	12.50	" " 7½ "
19	430	Chloramine-T 0.2 per cent	2.70	Sodium hypochlorite.	1.40	0.30	Lived.
20	600	" 2.0 " "	1.20	" "	6.40	1.10	"
21	502	" 2.0 " "	3.10	" "	16.00	3.20	Died in 3 hrs.
22	425	" 2.0 " "	5.30	" "	29.00	6.70	" " 1½ "
23	450	" 2.0 " "	11.25	" "	59.00	13.30	" " 1 hr.
24	525	Proflavine 0.1 per cent	6.55	Proflavine.	7.00	1.30	Lived.
25	525	" 0.1 " "	13.10	"	14.00	2.60	"
26	597	" 0.1 " "	30.00	"	30.00	5.00	Died in 72 hrs.
27	470	Dichloramine-T 0.5 per cent in bland oil	1.90	Sodium hypochlorite.	5.90	1.30	Lived.
28	470	" 0.5 " " " " "	5.90	" "	18.30	3.90	Died in 22 hrs.
29	550	" 5.0 " " " " "	2.20	" "	68.00	12 30	Lived.
30	498	" 5.0 " " " " "	6.20	" "	193.00	39.00	Died in 1½ hrs.
31	375	" 5.0 " " " " "	4.70	" "	146.00	39.00	" " 2 "
32	375	" 5.0 " " " " "	9.37	" "	292.00	78.00	" " 1 hr.

TABLE IV.

Summary.

Drug.	A. Mice, injected intraperitoneally.		B. Guinea pigs, injected intraperitoneally.		C. Guinea pigs, injected subcutaneously.	
	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Eucalyptol	0.15	0.05				
Brilliant green	0.15	0.05				
Mercurophen	1.50	0.50				
Mercuric chloride	2.50	0.50				
Chloramine-T	2.60	0.80	3.2	1.1		
Dichloramine-T	1.60 (?)		3.9 (?)			
	15.50	4.70	39.0	12.3		
Proflavine	25.00	5.00	5.0	2.6		
Hychlorite	12.00	12.00	6.2	3.0		
Dakin's hypochlorite	24.00	12.00	6.3	3.0	24.0	13.0
Commercial hypochlorite (Javelle water)	25.00	12.00				
Magnesium hypochlorite	25.00	12.00				
Iodine	100.00	35.00				
Phenol	50.00	50.00				

All the figures represent milligrams of antiseptic, or in the case of the chlorinated antiseptics, milligrams of sodium hypochlorite equivalent of the antiseptic, per 100 gm. of body weight.

Proflavine, hychlorite,[6] and Dakin's hypochlorite solution given intraperitoneally are all about two or three times as toxic per 100 gm. of body weight for the guinea pig as for the mouse. On the whole, however, the toxicity of the antiseptics follows about the same order in the two species of animal. When the great difference in the body weight of the mouse and the guinea pig is considered, the constancy of the lethal dose per unit of body weight is striking.

While it is, of course, not justifiable to calculate arbitrarily, on the basis of body weight alone, the fatal dose of these substances for man, it is interesting in this connection to note that if such a compu-

[6] Made by General Laboratories, Madison, Wisconsin.

tation could be considered valid the following amounts of certain of the antiseptics under the proper circumstances would constitute a fatal dose for a man weighing 70 kg.

0.14 cc. of equal parts of paraffin oil and eucalyptol (formerly considerably used as a solvent for dichloramine-T).

144 cc. of a 2 per cent solution of chloramine-T.

160 cc. of a 5 per cent solution of dichloramine-T in bland oil.

1,600 cc. of any of the hypochlorite solutions tested, having sodium hypochlorite titration of 0.5 per cent.

However, only a small amount of the antiseptic employed is absorbed from wound surfaces or from an abscess cavity, and little if any danger from constitutional effects would be expected from their employment in this way. When used in closed cavities, in the serous cavities of the body, or when sutured within a wound, these figures should, we believe, be kept in mind. This is especially the case in respect to eucalyptol used as a vehicle.

The drugs are tabulated in Table IV in the order of diminishing toxicity. It is interesting to note that the least toxic drugs that are efficiently bactericidal are the hypochlorite series and iodine.[7,8,9] The only two of the four hypochlorite solutions studied that are suitable for clinical use are Dakin's hypochlorite solution (in this case made from bleaching powder) and hychlorite. Of the other substances which vary somewhat in their greater toxicity for mice and guinea pigs, the most efficient are proflavine, dichloramine-T, chloramine-T, and possibly brilliant green. Mercurophen, mercuric chloride, and phenol can be disregarded as having too feeble disinfecting powers. Eucalyptol, the most toxic substance included in this study, is not recommended as a bactericidal agent, but merely as a solvent for dichloramine-T. Of these drugs, the only ones having appreciable solvent action on necrotic tissues, pus, etc., are the hypochlo-

[7] Dakin, H. D., and Dunham, E. K., Handbook of antiseptics, New York, 2nd edition, 1918.

[8] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

[9] Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

rites.[10, 11] Chloramine-T and the hypochlorites have also a destructive action on bacterial toxins.[12]

CONCLUSIONS.

1. The substances injected intraperitoneally into mice and guinea pigs arranged in the order of their decreasing toxicity are: eucalyptol and brilliant green; mercurophen; mercuric chloride and chloramine-T; dichloramine-T and proflavine; hychlorite, Dakin's hypochlorite, Javelle water, and magnesium hypochlorite; iodine and phenol.

2. Now that Dakin's bland solvent, chlorcosane, is available as a vehicle for dichloramine-T, eucalyptol should probably be discarded for this purpose because of its much greater toxicity.

3. Inasmuch as experienced surgeons do not approve of the injection of solutions of iodine and phenol into closed cavities, it would seem advisable not to use any of the antiseptics here discussed in this manner inasmuch as all exhibit a greater toxicity for mice and guinea pigs than the two chemicals first named.

4. The method of testing toxicity of antiseptics by subcutaneous injection is not satisfactory because exudation and subsequent sloughing reduce the rate of absorption and make uncertain the amount finally absorbed.

[10] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.
[11] Austin, J. H., and Taylor, H. D., *J. Exp. Med.*, 1918, xxvii, 627.
[12] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 375.